Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/11/89
Pharmacologist, Review Section I, TB-HFAS/HED (H7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D. YMI 7/13/89
Section Head, Review Section I, TB-HFAS/HED (H7509C)

DATA EVALUATION RECORD

I. Study Type: General Metabolism - Rat
Guideline: 85-1

Study Title: The Excretion and Metabolism of SENCOR by Rats

EPA Identification Numbers: EPA MRID No. 40255503
EPA Pesticde Chemical Code 101101
Caswell No. 33D
HED Project No. 9-027 A
Document No.

Sponsor: Mobay Corporation
Agricultural Chemicals Division
P.O. Box 4913
Hawthorn Road
Kansas City, MO 64120-0013

Testing Laboratory: Mobay Corporation
Agricultural Chemicals Division
Research and Development Department
Box 4913, Hawthorn Road
Kansas City, MO 64120

Study Number(s): Laboratory Project ID: SE4R
Mobay AG Chem No. 94605

Study Date(s): June 25, 1987

Study Author(s): K.S. Cain, C.M. Hanlon, J.B. Lane

Test Material: ^{14}C - SENCOR
Vial No. C-380B
Specific Activity = 20.8 mCi/mmol
Purity = 98.4% to 99.4%
Unlabeled SENCOR
Lot # 51025
Purity = 99.0%

Test Animals: Young adult male and female Wistar rats
(Rattus norvegicus)
Supplier: Charles River Breeding Laboratories,
Inc., Boston, MA

II. Materials and Methods: A copy of the "Materials and Methods" section from the investigators report is appended. The comments and highlights on these "materials and methods" are as follows.

Animals were fasted 24 hours prior to radiolabelled compound administration, water was available. The animals received food and water, ad libitum, after administration of the test compound.

Animals weighed 146 to 263 gms at time of dosing and were approximately 9 weeks of age. They were acclimated 7 days prior to dosing. Separate plastic metabolism cages were used after dosing (except for preliminary study where glass cages were used).

Dosing solutions were freshly prepared for each test group. The investigators followed the recommendation in the Pesticide Assessment Guidelines for choice of test groups: a low dose group treated with a single dose corresponding to the NOEL; a high dose group receiving a single administration; a repeated dose group receiving unlabelled compound daily for 14 days (low dose level) followed by a single labelled dose. Oral dosing was the chosen route, they did not employ intravenous dosing since SENCOR (Metribuzin) does not have adequate water solubility.

Preliminary studies were conducted on the volatility of SENCOR and its metabolites. Two female rats were used. They were treated with a 5.0 mg/kg single dose and placed in all glass metabolism cages. Urine, feces and expired air were collected. Urine and feces collection times were 8, 12, 24, 48, 72, and 96 hours. Urine was collected under various conditions, see attached "materials and methods" for additional information.

For the primary study, urine and feces were collected at 8, 12, 24, 48, 72 and 96 hours. Urine was collected in the presence of 5 ml isopropanol (based on results of preliminary study). Urine volumes were measured. Samples of urine and feces were handled for analysis as described in the attached "materials and methods."

Rats were anesthetized 96 hours post treatment. Blood was collected from the descending aorta. The liver, heart, gonads, gastrointestinal tract, spleen and kidneys were removed. Further, the brain was removed and samples of fat, bone and muscle were taken. Weights of all samples were recorded and treated as described in the attached "materials and methods."

Cages were rinsed with 150 ml of methanol followed by 150 ml of 2N HCl. The samples were then analyzed.

Samples of urine were used for metabolite isolation and identification. The investigators prepared separate composite samples of each sex for 0 through 48 hours for the low dose groups and 0 through 72 hours for the high dose group. Aqueous and organic extractions including enzymatic and acid hydrolysis treated samples were analyzed by thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) or gas chromatography (GC). GC-Mass Spectromety (GC-MS) was also carried out on some samples. See attached "materials and methods" for more detail.

Sample of feces were also used for metabolite isolation and identification. The investigators used separate composite samples for each sex for the single dose low and high dose groups at 0 to 48 or 72 hours, respectively. For the mulitple dose animals, composite samples of females of 0 to 72 hours and for males of 0 to 48 hours. The samples were treated in a similar manner as with the urine samples.

A signed statement of "no claim of confidentiality" was included.

A signed "Good Laboratory Practice Certification" was included.

A signed "Certificate of Authenticity" was included.

III. Results

A. Preliminary Study

The investigators found "insignificant amounts of radioactivity (<0.1% of administered dose)" in expired air, therefore, expired air was not collected during the primary study (as allowed by the EPA Guidelines).

The investigators found that approximately 60.4% of the label was excreted in the feces and 38.4% in the urine. Further, they found that greater than 95% of the administered dose was excreted by 96 hours, therefore, they limited the primary study to this time frame.

Urine was collected under 2 conditions, either at 0°C or in the presence of isopropyl alcohol. Both were intended to inhibit microbial activity. No major differences were noted, so the investigators used isopropyl alcohol based on "ease of handling".

B. Primary Study

1. Clinical Observations

The high dose group (500 mg/kg) presented with labored breathing and either rapid blinking or closed eyes. Some animals were reported to "tremble and then become very still". Two female rats died, one on day 2 and the other on day 3. The investigators used two replacement females. No Male rats were reported to have died. In the low dose group (5 mg/kg), one female was eliminated due to gavage error and one male from diarrhea (before and after treatment). No animals were replaced, however, as there were still 5 animals per sex left, which meets the EPA Guidelines recommendation.

No clinical observations were reported for the repeated dose group.

2. Radiolabel Recovery

The following table extracted from the investigators report presents the label recovered.

Percent of Administered Radioactivity

	Group B		Group C		Group D	
	Male	Female	Male	Female	Male	Female
Urine	37.6	37.7	40.0	43.4	27.3	32.2
Feces	60.5	61.0	59.1	55.8	71.5	66.3
Blood	0.1	0.2	0.1	0.1	0.2	0.1
Tissues	0.2	0.2	0.2	0.1	0.2	0.3
Carcass	0.6	0.4	0.2	0.2	0.3	0.4
Cage Rinse	1.0	0.5	0.4	0.4	0.5	0.7
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0
Actual % of Radiolabel Recovered	106.1	106.1	95.2	95.8	104.7	102.5

Mean Recovery = 101.7

Standard Deviation = 5.0

Data extracted from Table IV, Mobay Ag Chem# 94605.

The investigators "normalized" the above data for ease of reporting. The major route of excretion was the feces, ranging from 55.8 to 71.5% of administered label. The urine excretion was slightly less varying from 27.3 to 43.4%.

3. Radiolabel Excretion

The investigators stated that "no significant differences were detected in the rates and routes of ^{14}C-elimination between male and female rats", this pertains to the total excretion and the previous table supports this statement. Little residual radiolabel was found in the blood, tissues, carcass and cage rinse. The above results are similar to an earlier study. Mobay AG Chem # 33366 conducted in 1972 (DER attached).

The investigators further stated that there were "no significant differences in the rates and routes of radiocarbon elimination between male and female rats" in either the low or high dose single administration groups. This can be seen, on attached Figures 4 and 5 from the investigators report. The excretion levels appear to plateau at approximately 48 hours for the low dose and at approximately 72 hours for the high dose.

The investigators stated that the repeated dose group showed a difference in the rate of elimination between males and females. They stated that: "The percent of radiocarbon eliminated via the urine reached a plateau by 48 hours in the male and female rats". And further, "However, in the feces, the male rats' elimination of radiocarbon reached a plateau by 48 hours and the female rats' feces elimination of radioactivity did not reach a plateau until 72 hours post-administration of ^{14}C-SENCOR". Figure 6 from the investigators report, however, shows that elimination in both urine and feces reached a plateau at slightly greater than 70 hours. The group mean data also seem to support this 70 plus hours plateau, although one can stretch the point and say that the urine levels began the peak at 48 hours.

4. Tissue Distribution

Attached combined Tables V and VI presents the percent of and concentration of dose remaining in various tissues. As can be seen on the Tables, very little of the administered dose (in%) remained in the tissues and blood with the residual carcass containing the highest levels. Low dose group levels in ppm were found in very low amounts, with slightly greater amounts in ppm found in the high dose group.

5. Metabolite Identification

The investigators used pooled samples of urine and feces for metabolite isolation and identification by TLC, HPLC and GC.

a. Urine Metabolite Analysis

The investigators subjected the urine samples to organic extraction (after acidifying the samples to pH 2) with methanol extraction of the aqueous phase. They found 16 metabolites, 12 of which could be identified, see attached Table IX. No apparent differences were noted in percent distribution between sexes or among treatment groups. Very small amounts of the parent compound were recovered. According to the investigators, the most prevalent metabolites were the DA and DA-N-Ac-Cys followed by DADK, DK, t-BuOH-SENCOR, t-BuOH-DADK, t-BuOH-DA, t-BuOH-DK, Butylthione, SENCOR-N-Ac-Cys and 3-amino-DA.

Further analysis of Unknown 1 indicated that it may be comprised of more than one conjugated metabolite of SENCOR (metribuzin).

Treatment with beta-glucuronidase did not release any conjugated material in the urine sample. The investigators, therefore, deduced that the 4 unknowns were not beta-glucuronide conjugates.

The investigators included an open literature paper (as an appendix) that supports their findings (attached).

b. Fecal Metabolite Analysis

The pooled fecal material samples were also treated by organic solvent extraction with an extraction of the aqueous phase. The majority of the metabolites were found in the aqueous phase. After the solvent extraction, the fecal solids were treated by acid hydrolysis and then extraction of the filtrate. Attached Table XI presents the distribution of metabolites in percent. No specific differences were noted between sexes or among dose groups except for higher levels of unknown 1 in Groups C and D. The most prevalent metabolites were DA-N-Ac-Cys (this was also the most prevalent in the urine samples) and DADK followed by parent SENCOR, DA, DK, t-BuOH-SENCOR, t-BuOH-DADK, t-BuOH-DA, t-BuOH-DK, SENCOR-N-Ac-Cy and 3-amino-DA.

The unknowns were further treated with beta-glucuronidase, however, no conjugated materials were released. Therefore, the investigators concluded that the 4 unknowns were not beta-glucuronide conjugates of SENCOR (metribuzin) metabolites.

IV. Discussion and Conclusions

The absorption of metribuzin (SENCOR) could not be determined since the low water solubility of the compound precluded the use of an IV dosed group.

Excretion data indicated that radiolabeled metribuzin is rapidly excreted in the urine and feces (observed at 8 hours) and reached a plateau at 48 hours for the single dosed groups (low and high), except the high dose female feces which reached a plateau at 72 hours. The investigators found 27.3 to 43.4% of the label in the urine and 55.8 to 71.5% of the label in the feces at 96 hours. Pilot studies determined that negligible amounts of label were found in the expired air, therefore, it was not collected in this primary study. The high dose group excreted the greatest amount of the label in the feces with the least amount of label, of all 3 test groups, in the urine.

Blood levels at various time points were not determined, however, very small amounts were found at 96 hours (sacrifice) in the low dose and slightly higher levels in the high dose at sacrifice.

No specific differences in tissue levels were noted in the low dose groups. The high dose group had higher tissue levels, which would be expected, with the GI tract having considerably higher levels.

The investigators identified metabolites in both the urine and feces with the most prevalent metabolite being DA-N-Ac-Cys.

The investigators proposed a metabolic pathway, for Metribuzin in rats, see attached Figure 12. They further stated that:

> "The metabolism of SENCOR in rats appears to involve deamination, dethioalkylation, hydroxylation of the t-butyl side chain and conjugation.
>
> The appearances of SENCOR-N-Ac-Cys and DA-N-Ac-Cys suggest SENCOR is undergoing conjugation with glutathione. These glutathione-conjugates are subsequently processed through the mercapturic acid pathway. The n-acetyl cysteine conjugates can be excreted either in the urine or the bile, explaining their presence in both the urine and feces samples of the rats. The detection of Butylthione in the urine could be a result of cleavage of the acetyl cysteine groups in the kidneys".

V. Core Classification: Acceptable

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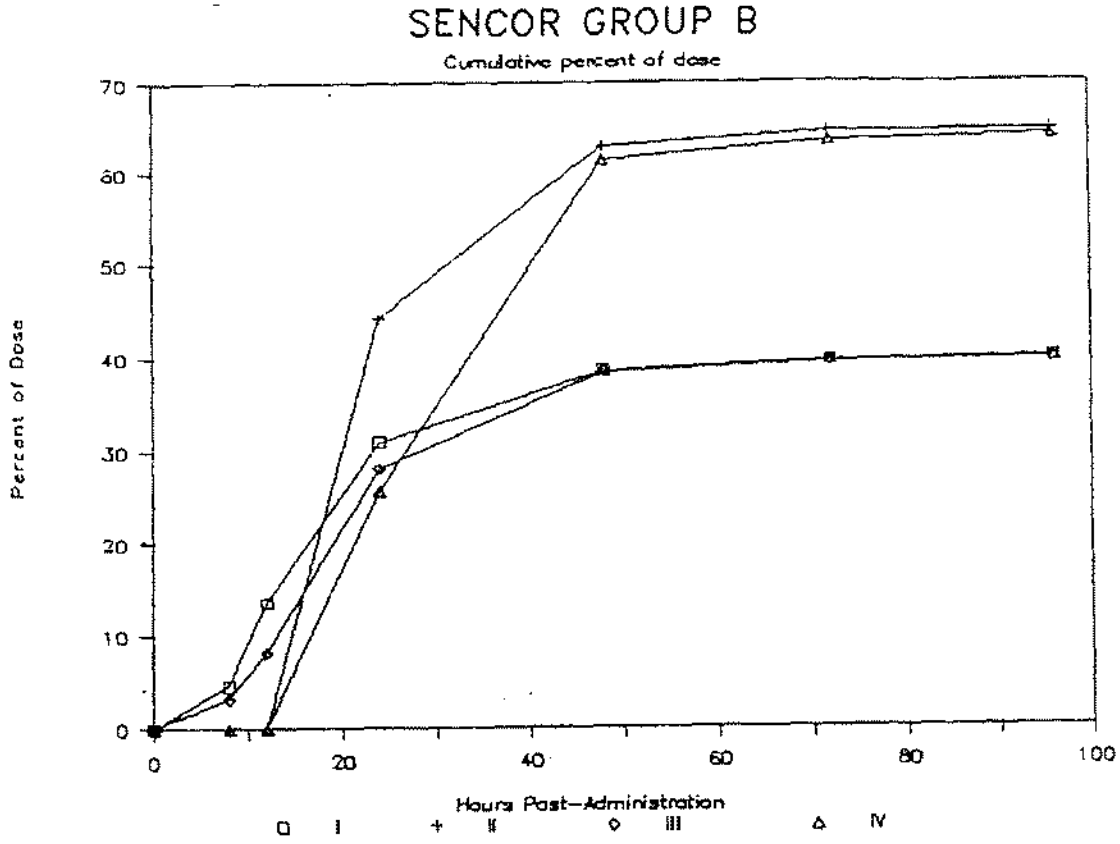

Figure 4. Elimination of radioactivity from Group B rats treated orally with 5 mg/kg ^{14}C-SENCOR. Tabular data are presented in Appendix B. I = Female Urine, II = Female Feces, III = Male Urine, IV = Male Feces.

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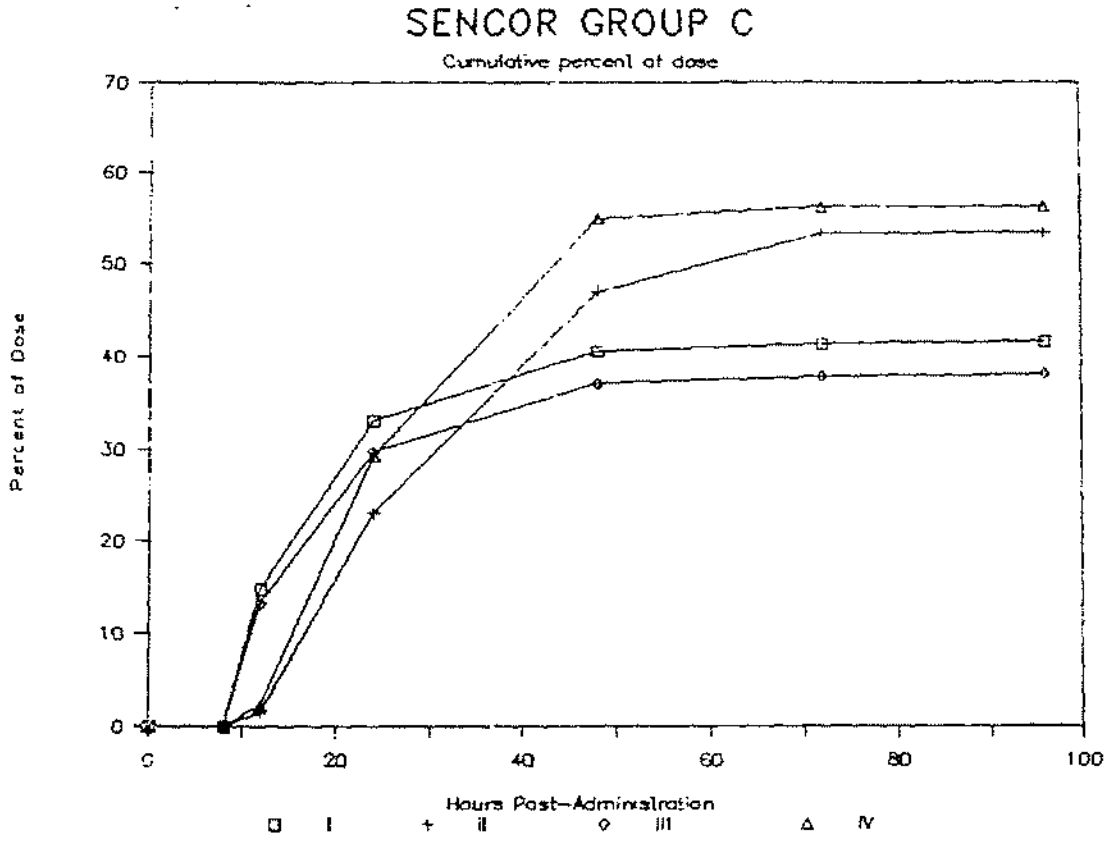

Figure 5. Elimination of radioactivity from Group D rats treated orally with 500 mg/kg ^{14}C-SENCOR. Tabular data are presented in Appendix B. I = Female Urine, II = Female Feces, III = Male Urine, IV = Male Feces.

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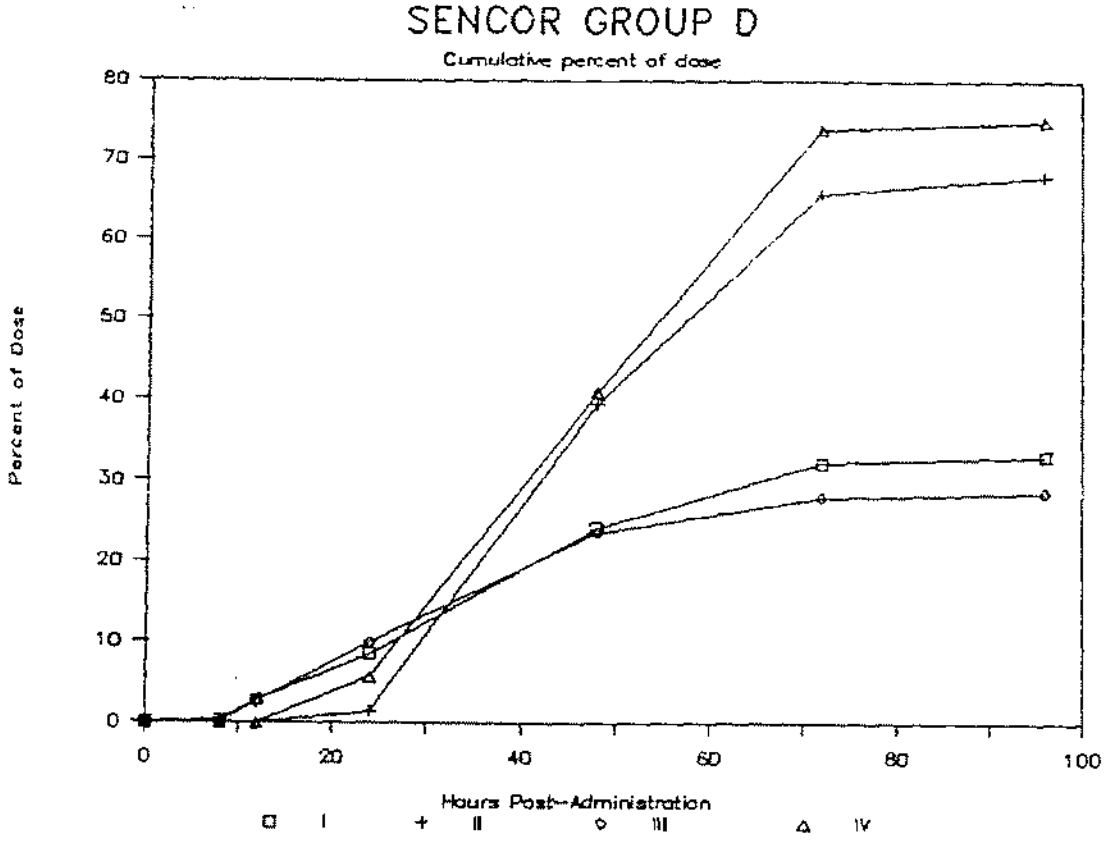

Figure 6. Elimination of radioactivity from Group C rats treated orally for 14 days with ^{12}C-SENCOR (5 mg/kg) followed by a single oral dose (5 mg/kg) of ^{14}C-SENCOR. Tabular data are presented in Appendix B. I = Female Urine, II = Female Feces, III = Male Urine, IV = Male Feces.

Tables V and VI Distribution of Radioactivity in Tissues after 96 Hours in percent and ppm of administered dose

	% Group B		ppm Group B		% Group C		ppm Group C		% Group D		ppm Group D	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Blood	0.18	0.12	0.230	0.160	0.15	0.14	0.247	0.172	0.13	0.16	25.902	18.138
Bone	0.00	0.00	0.008	0.009	0.00	0.00	0.008	0.007	0.00	0.00	1.155	0.777
Brain	0.00	0.00	0.009	0.008	0.00	0.00	0.008	0.007	0.00	0.00	1.480	0.826
GI Tract	0.04	0.06	0.013	0.015	0.04	0.05	0.017	0.019	0.11	0.14	3.302	4.251
Fat	0.00	0.00	0.011	0.009	0.00	0.00	0.014	0.010	0.00	0.00	1.618	0.994
Gonads	0.00	0.00	0.014	0.008	0.00	0.00	0.013	0.008	0.00	0.00	1.562	0.752
Heart	0.00	0.00	0.043	0.036	0.00	0.00	0.042	0.027	0.00	0.00	6.150	3.265
Kidney	0.02	0.01	0.081	0.065	0.02	0.01	0.105	0.060	0.01	0.01	7.993	4.964
Liver	0.07	0.08	0.071	0.078	0.07	0.07	0.076	0.073	0.09	0.08	7.364	5.712
Lung	0.01	0.01	0.097	0.074	0.01	0.01	0.093	0.063	0.01	0.01	9.053	5.796
Muscle	0.00	0.00	0.006	0.006	0.00	0.00	0.007	0.006	0.01	0.00	1.187	0.693
Spleen	0.00	0.00	0.055	0.038	0.00	0.00	0.076	0.040	0.00	0.00	6.304	4.070
Carcass	0.39	0.53	0.027	0.032	0.20	0.21	0.014	0.014	0.36	0.25	2.735	1.671

Group B = low dose, single administration

Group C = repeated administration of unlabeled Metribuzin followed by single low dose administration

Group D = high dose, single administration

Data extracted from Mobay AG Chem # 94605

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TABLE IX

SENCOR Rat Metabolism
Percent Distribution of Metabolites[1]
Detected in Urine

	Urine Percent of Dose					
	Group B		Group C		Group D	
	Female	Male	Female	Male	Female	Male
Butylthione	0.1	0.0	0.1	0.0	0.8	0.5
SENCOR	0.7	1.2	1.0	0.6	0.5	0.5
DADK	3.3	2.6	3.2	4.2	2.2	1.0
OA	4.8	7.3	8.6	8.4	1.5	2.1
OK	2.0	1.6	1.8	2.0	4.3	1.8
t-BuOH-SENCOR	0.2	0.3	0.0	0.0	0.4	0.2
t-BuOH-DAOK	1.5	1.5	2.1	1.9	2.1	1.6
t-BuOH-DA	1.3	1.6	2.1	0.9	0.6	0.8
t-BuOH-DK	1.6	2.3	1.8	1.7	3.4	1.0
SENCOR-N-Ac-Cys	0.1	0.4	0.1	0.3	0.3	0.7
DA-N-Ac-Cys	6.7	7.3	6.7	7.8	6.0	6.4
3-Amino-OA	1.1	1.1	1.5	2.0	1.6	1.1
Subtotal	23.4	27.1	28.9	29.8	23.6	17.6
Unk 1	4.4	2.5	4.0	4.4	6.4	5.0
Unk 2	0.4	0.3	0.5	0.3	0.5	0.7
Unk 3	0.0	0.0	0.0	0.0	0.7	0.4
Unk 6[2]	0.4	0.6	0.7	0.7	0.7	0.3
Diffuse[3]	0.0	0.0	0.2	0.4	0.0	0.0
Origin	3.7	3.2	6.1	5.0	2.8	2.6
Total Percent of Dose	32.2	33.7	40.4	40.8	34.7	26.5

[1]See Figure 1 for structures. Values expressed as percents of total ^{14}C-residue in tissues.
[2]Unk 4 and 5 only seen in feces.
[3]Radioactivity not associated with any identified standard.

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TABLE X1

SENCOR Rat Metabolism
Percent Distribution of Metabolites[1]
Detected in Feces

	Feces Percent of Dose					
	Group B		Group C		Group D	
	Female	Male	Female	Male	Female	Male
SENCOR	1.0	1.2	1.5	1.7	1.8	1.9
DADK	5.1	5.5	6.2	6.1	4.8	3.8
OA	1.1	0.9	0.6	0.6	2.6	2.4
DK	4.3	3.7	2.6	3.4	1.8	1.3
t-BuOH-SENCOR	0.3	0.1	0.8	0.6	0.3	0.3
t-BuOH-DADK	2.1	1.5	2.1	2.0	4.9	3.5
t-BuOH-DA	0.9	0.7	0.4	0.4	0.2	0.1
t-BuOH-DK	1.9	1.4	0.6	1.6	0.3	1.7
SENCOR-N-Ac-Cys	0.9	0.8	1.4	0.6	0.0	0.0
DA-N-Ac-Cys	9.4	9.1	6.2	6.5	17.7	15.3
3-Amino-DA	1.9	1.7	2.6	3.3	3.5	3.9
Subtotal	29.0	26.7	24.9	26.8	37.8	34.3
Unk 1	1.1	1.0	7.5	8.6	2.8	4.9
Unk 4[2]	4.5	3.5	2.3	2.8	0.0	0.0
Unk 5	0.1	0.0	0.2	0.2	0.1	0.1
Unk 6	1.0	1.0	0.2	0.2	0.2	0.3
Diffuse[3]	0.2	0.2	0.7	0.8	0.1	0.2
Origin	4.1	3.3	4.0	4.8	3.8	3.3
Total Percent of Dose	40.2	35.7	39.9	44.3	44.8	43.1

[1]See Figure 1 for structures. Values expressed as percents of total ^{14}C-residue in tissues.
[2]Unk 2 and 3 only seen in urine.
[3]Radioactivity not associated with any identified standard.

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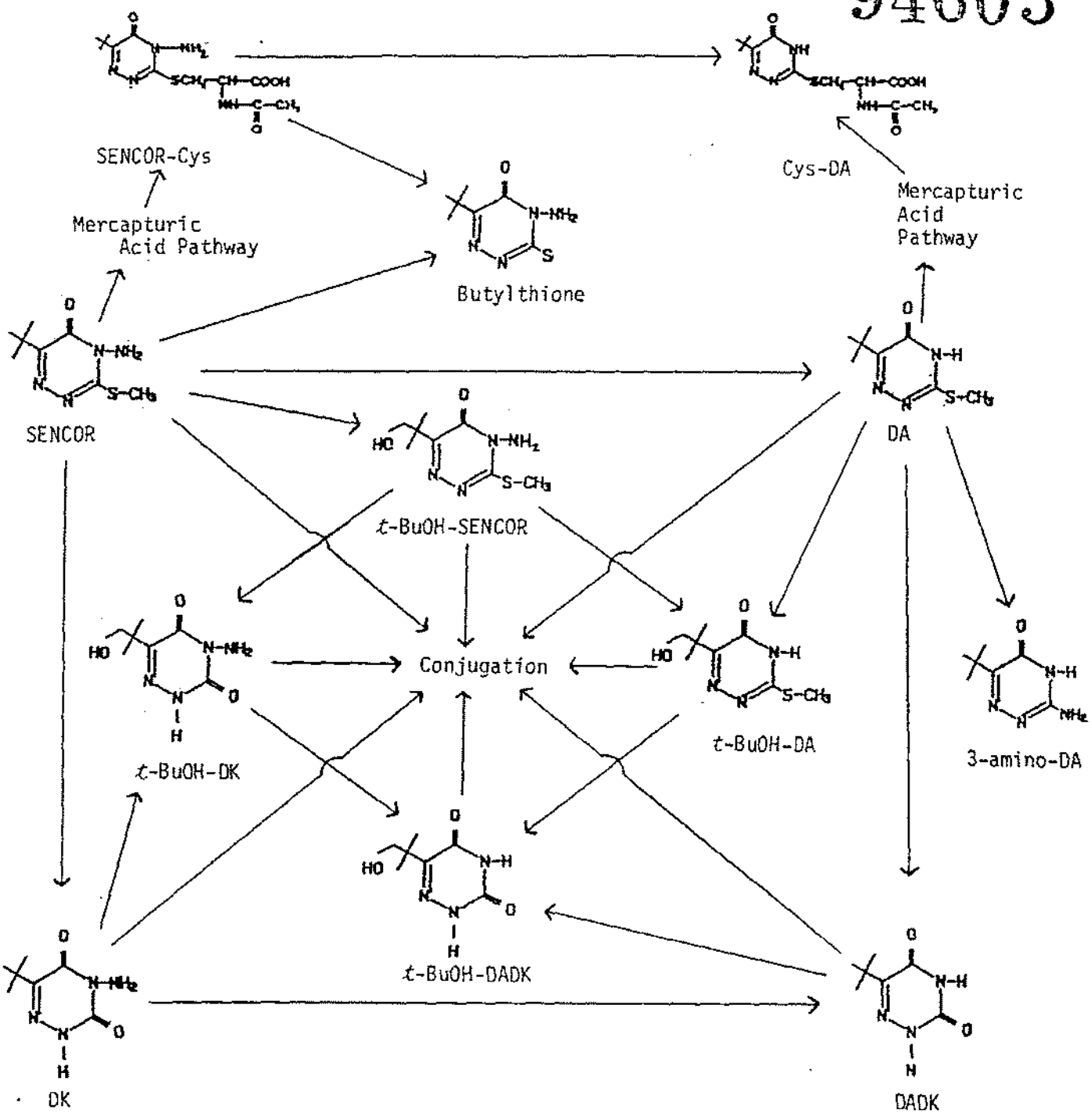

Figure 12. Proposed metabolic pathway of SENCOR in rats.

INTRODUCTION

The excretion and metabolism of SENCOR, (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5H)-one) by male and female Wistar rats was investigated.

EXPERIMENTAL

Animals were treated and maintained at the Mobay Toxicology Department facility, Stanley Kansas. The laboratory research and sample analyses were conducted at the Biochemistry Research Laboratories of Mobay Corporation, Agricultural Chemicals Division at Kansas City, Missouri and Stanley, Kansas. Studies were conducted from November 1985 to May 1987.

Materials

^{14}C-SENCOR (vial No. C-380B), specific activity 20.8 mCi/mmol, with a ^{12}C:^{13}C:^{14}C ratio of 1:1:1 at the 5 position of the ring, was synthesized by Poje[1]. Radiochemical purities, determined by thin-layer chromatography (TLC, HEDH TLC system, see Table I) were 99.4% for the low dose preparation, 98.4% for the repeat dose preparation and 98.9% for the high dose preparation. Unlabeled SENCOR, Lot #51025[2], used for admixture in the high dose preparation, had a chemical purity of 99.0%.

The structures and chromatographic characteristics of SENCOR and related standards are shown in Figure I and Table I. These standards were prepared in the research laboratories of Mobay Corporation, Kansas City, MO and Bayer AG, Federal Republic of Germany. Their identities were confirmed by gas chromatography/mass spectrometry (GC/MS).

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Animal Handling

Young adult (male and female) Wistar rats (*Rattus norvegicus*) were obtained from Charles River Breeding Laboratories, Inc., Boston, MA. The rats were acclimated for at least 7 days before administration of SENCOR.

The rats were fasted for 24 hours before ^{14}C-dose administration, and were allowed food *ad libitum* after administration of the test compound. Drinking water was available to the animals at all times.

At the time of ^{14}C-dosing the individual rats weighed 146 to 263 g. Their approximate ages were 9 weeks. The dose for each individual rat was based on the average weight of all the animals in the group, or in the case of the repeat dose level the average weight according to sex. Individual body weights were within 16% of the group mean. Rats were treated with 2 to 5 μl of dose solution per gram body weight. Each dose was administered orally via a stainless steel gavage dosing needle on a serological syringe. After dosing, the rats were placed in separate Nalgene metabolism cages which allowed for the separate collection of urine and feces with the exception of the preliminary study where the rats were housed in glass cages.

Dose Preparation and Administration

A new dosing solution was prepared for each group of rats. SENCOR-5-^{14}C (stored at room temperature in benzene) was prepared for dosing by evaporating the solvent under a gentle stream of nitrogen. The ^{14}C-SENCOR was then redissolved in ethanol:propylene glycol (1:1). Dosing solutions of unlabeled SENCOR were prepared as described for the ^{14}C-SENCOR.

As shown in Table II, 3 dose groups were selected according to the EPA guidelines. The low dose experiment (Group B) rats were treated orally with a single 5 mg/kg dose of ^{14}C-SENCOR which corresponded to the no-effect level seen in a previous study[3]. The high dose experiment (Group D) rats received a single oral dose of radiolabeled SENCOR at 500 mg/kg. The repeat dose experiment (Group C) rats were dosed daily at 5 mg/kg (orally) for 14 consecutive days with unlabeled SENCOR followed by a single oral 5 mg/kg radiolabeled dose. Table II is a summary of all the groups of rats and the actual amount of ^{14}C-SENCOR administered to each group. The weights of the individual rats are listed in Appendix A.

Concerning the intravenous dosing of rats (EPA group A), the EPA Guidelines state, "If it is not possible to dissolve the test substance in physiological saline or water, this group may be omitted[4]." The rats were dosed at 5 μl of dosing solution per gram of body weight which is approximately 8-10 % of their total blood volume.

$$(5\ \text{ppm})\quad \frac{5\ \mu g\ \text{SENCOR}}{g\ \text{body wt}} \times \frac{g\ \text{body wt}}{5\ \mu l\ \text{soln}} = \frac{1\ \mu g}{\mu l} \text{ or } \frac{1\ mg}{ml} = \frac{1\ mg}{g}$$

$$= 1000\ \text{ppm}$$

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SENCOR has a water solubility of 980 ppm so it was not possible to administer the compound by intravenous injection at the required dose level (5ppm).

Preliminary Studies

To determine the volatility of SENCOR and its metabolic products, a preliminary study was conducted with two female rats. Both rats were treated with 5.0 mg/kg of ^{14}C-SENCOR and placed in individual, all glass Roth metabolism cages which allowed separate collection of urine, feces and expired gases. Air was drawn through the cages at a rate of approximately 235 ml/min. Incoming air passed through separate towers of Drierite (removes water) and Ascarite (CO_2 scrubber) prior to entering the cages. Expired gases were passed through 200 ml of 10% sodium hydroxide (NaOH) and sampled at sacrifice. The urine from rat #A was collected in a container kept on ice during the sampling period and the urine from rat #B was collected in the presence of 5 ml isopropyl alcohol (IPA). Urine and feces were collected at 8, 12, 24, 48, 72 and 96 hours.

Sample Collection and Quantitative Analysis

Urine and feces were collected from rats (Groups B, C and D) at the intervals indicated in Table III. Urine was collected in the presence of 5 ml isopropanol. Urine volumes were measured and duplicate aliquots from each sample were quantitated by liquid scintillation counting (LSC) using a Packard Tri-Carb Model 460 liquid scintillation spectrometer with automatic external standardization. Fecal samples from each rat at each sampling interval were homogenized with a small metal spatula and water was added, if needed, to produce a homogeneous mixture. Triplicate aliquots (100 to 350 mg) were oxidized to $^{14}CO_2$ in a Packard Model 306 sample oxidizer and radioassayed by LSC.

Ninety six hours after administration of the ^{14}C-SENCOR dose the rats were anesthetized with halothane until eye blinking ceased and respiration slowed. A longitudinal incision was made to open the peritoneum. Approximately I to 2 ml of Heparin in water (Sigma Chemicals, 2 mg/ml) was added to the peritoneal cavity and as much blood as possible was collected from the descending aorta. Liver, lungs, heart, gonads, gastrointestinal (GI) tract, spleen and kidneys were removed. The skull was opened and the brain was removed through the temporal cavity. Samples of fat, bone and muscle were also taken. Weights of all tissues and total body weight were recorded at the time of sacrifice. The tissues were rinsed with water, if necessary, to remove excess surface blood. Tissues, GI tracts and carcasses were frozen until processed.

Tissues, excluding the GI tracts, were minced using scissors. Individual GI tracts, with contents intact, were processed by chopping with dry ice in an Omni-mixer (Ivan Sorvall, Inc). Carcasses were individually chopped with dry ice in a Hobart food chopper. Triplicate aliquots of all tissues were oxidized to $^{14}CO_2$ and quantitated by LSC.

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After sacrifice of the animals, the metabolism cages were rinsed with 150 ml of methanol followed by 150 ml of 2 *N* aqueous hydrochloric acid (HCl) solution. Triplicate aliquots of each rinsing solution were analyzed for total radiocarbon content by LSC.

Material balance and tissue residue data were compiled with a Lotus 1,2,3 spreadsheet. Appendices B through E contain examples of these spreadsheets.

Metabolite Isolation and Identification

Sample Preparation. Separate composite urine samples of each sex were prepared for Groups B and C by combining one third of the total urine collected per individual rat at 0 through 48 hour post-administration. Composite urine samples for each sex in Group D were prepared by combining one third of the total urine collected per individual rat from 0 to 72 hours post-administration.

Separate composite feces samples were prepared for each sex in Groups B and D by combining one half of the total feces collected per individual rat at 0 to 48 hours or 72 hours, respectively. Composite feces for Group C females were prepared by combining one half of the total feces from 0 to 72 hours and for Group C males, one half of the total feces from 0 to 48 hours.

Urine Sample Extraction and Analysis. Composite urine samples from each group and sex were extracted individually using the procedure shown in Figure 2. An equal volume of saturated aqueous sodium chloride (NaCl) was added to the urine which was then acidified to pH 2 with 6 *N* HCl. The urine was extracted 3 times with isopropyl ether:ethanol (IPE:EtOH, 3:1). The Urine Organic phases were combined, drained through sodium sulfate to remove traces of water and concentrated. The organic phase was then quantitated by LSC and analyzed by TLC, high pressure liquid chromatography (HPLC) and gas chromatography (GC). The Urine Aqueous phase was concentrated by rotary evaporation at 37°C and eluted through an XAD-2 resin (Sigma Chemicals) mini column (described later) which was washed with H_2O and then with methanol (MeOH). Both the H_2O and MeOH fractions were radioassayed by LSC. The methanol fraction was analyzed by TLC.

Feces Sample Extraction and Analysis. The extraction scheme for analysis of composite feces samples from each group and sex is presented in Figure 3. Composite feces were extracted individually in MeOH with a Polytron tissuemizer (Brinkmann Instruments) and filtered through Whatman filter paper, #42. The solids were then extracted with ethyl acetate:MeOH (7:3) and filtered again. The solids were extracted the final time with MeOH:H_2O (7:3) and filtered. The combined filtrates were evaporated to an aqueous phase and extracted three time with dichloromethane:acetonitrile (DCM:ACN, 2:1). The organic phase (Feces organic) was concentrated and analyzed by TLC, HPLC and GC. The aqueous filtrate (Feces aqueous) was applied to a column (described later) filled with XAD-2 resin, and eluted sequentially with H_2O, MeOH and MeOH:6 *N* HCl (1:1). All fractions were analyzed by TLC. The Feces organic and fractions of the Feces aqueous were radioassayed by LSC.

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The extracted feces solids were refluxed overnight in 6 *N* HCl:MeOH (1:1) and filtered. The filtrate was evaporated to the aqueous phase and adjusted to pH 2 with NaOH. The feces filtrate was diluted with an equal volume of saturated aqueous NaCl solution and then extracted 3 times with IPE:EtOH (3:1). The organic phases (designated Feces acid organic) were combined and concentrated prior to TLC and HPLC analysis, and LSC quantitation. The aqueous phase (designated Feces acid aqueous) was concentrated by rotary evaporation and quantitated by LSC. Aliquots of the hydrolyzed feces solids were oxidized to $^{14}CO_2$ and radioassayed by LSC.

Chromatography. Various chromatographic systems are listed in Table I. Metabolite analyses were performed by thin layer chromatography on silica gel 60 F_{254} plates (0.25 mm thickness, E. Merck). As shown in Table 1, the solvent systems used were : HEDH, hexane:ethylene glycol dimethyl ether: dichloromethane:acetic acid (60:40:10:1); IAW, isopropyl alcohol:ammonium hydroxide:water (8:1:1) and BEH, benzene:ethyl acetate:acetic acid (50:10:1). Radiolabeled SENCOR and metabolic products were located by autoradiography (Kodak film XAR-5). Unlabeled authentic standards were located by visualization under ultraviolet light (254 nm). Radioactive areas of interest were scraped from TLC plates, eluted off the silica gel with MeOH and quantitated by LSC.

Analyses by HPLC were performed on a Zorbax C_8 reverse phase column (4.6 mm x 25 cm, 5 μ). Two main mobile phases were used; one was a phosphate buffer/ MeOH system and the other was an ACN/H_2O/HOAc (HOAc = acetic acid) system. The solvent flow rate was I ml/min. HPLC system A was a linear gradient of 100% 0.02 *M* potassium phosphate buffer (pH 6.5) to 100% MeOH over 50 minutes. HPLC system B was an isocratic composition of ACN:H_2O:HOAc (875:325:2). HPLC system C was a linear gradient of ACN:H_2O:HOAc (25:225:1) to ACN:H_2O:HOAc (150:100:1) over 40 minutes. HPLC system D was a linear gradient from H_2O:HOAc (250:1) to ACN:HOAc (250:1) over 30 minutes. The wavelengths monitored in the systems were either 275 nm or 280 nm depending on the instrument. Two different HPLC systems were used. Detection was monitored either with a Kratos Spectroflow 773 variable wavelength UV detector and a Nuclear Enterprise Isoflow radioactivity monitor equipped with a 200 μl flow cell or a Hewlett Packard variable wavelength detector and a Berthold radioactivity monitor (Model LB 505).

Gas chromatography conditions were as follows:

A Hewlett Packard 5890A gas chromatograph was equipped with an electron capture detector fitted with a 15 meter wide bore capillary column, 3-μm film of OV 1701 (Quadrex, Thikote). The temperature was programmed from 180° to 250°C at 10° per minute with the detector set at 300°C and the injector 240°C.

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A Hewlett Packard 5730A gas chromatograph was equipped with an electron capture detector fitted with a 15 meter wide bore capillary column, 1 μm film of OB 17 (J + W Co). The temperature was programmed from 200° to 250°C at 4° per minute with the detector temperature set at 300°C and the injector temperature 250°C. The carrier gas was helium at 2 ml/min and the makeup gas was a mixture of methane:argon (5%) at 58 ml/min.

XAD-2 resin columns. Open ended, gravity flow columns packed with XAD-2 resin (Sigma Chemical) were used for cleanup of various aqueous fractions. New resin was used for each column. The resin was cleaned prior to use twice with MeOH and twice with acetone by stirring the resin in the solvent for several minutes and vacuum filtering. The cleaned resin (approximately 40-50 g dry weight) was packed in the open glass columns (1.8 cm i.d. x 25 cm) as a MeOH slurry and washed with approximately 500 ml fresh MeOH. The columns were then washed with 500 ml water prior to sample application. The water was allowed to drain until it reached the top of the resin bed prior to sample application. The mini columns used with the Urine Aqueous phases (1.8 cm x 15 cm) contained only 10 to 15 g of resin.

Prior to column cleanup, the samples were concentrated, applying 10 ml or less to each column. The samples were allowed to drain to the top of the resin bed before the eluting solvents were added. The columns were washed with 75 to 125 ml of water, until color no longer eluted from the column. The columns were next washed with 100 to 150 ml MeOH until no more color was eluted and the final eluting solvent was MeOH:6 *N* HCl (1:1), 100 to 150 ml, again until all color was washed through the column. The radioactivity in each fraction was quantitated by LSC. The MeOH and MeOH-HCl fractions were concentrated by rotary evaporation and analyzed by TLC, HPLC and GC.

Enzyme hydrolysis. Enzyme hydrolysis with β-glucuronidase was performed on composite urine and feces samples. The reactions were carried out in sample vials from Sigma Chemicals Company that contained the enzyme, (from bacteria, 1000 units) and buffer salts for 10 ml of a 4 mM potassium phosphate buffer, pH 6.8. Positive controls were run previous to the samples, ensuring that the enzymes were active. The sample (previously adjusted to a pH 6.8) and H_2O were added to the sample vials yielding a final volume of 10 ml. The mixture was incubated overnight at 37°C. Control samples included the urine or feces sample, H_2O and potassium phosphate buffer (pH 6.8) excluding the enzyme. Hydrolysis products were extracted into IPE:EtOH and examined by TLC.

Acid Hydrolysis. Isolated unknown samples of urine composites were concentrated to 1 to 2 ml in MeOH and incubated with an equal amount of 6 *N* HCl for 1 hour at 60°C. Hydrolysis products were examined by TLC.

Mass Spectral Analysis. A Hewlett Packard 5995 Mass Spectrometer (electron impact, 70 eV) coupled with a gas chromatograph was used for mass spectral analyses. The GC was equipped with a 12 meter methyl silicone (0.2 mm ID) column.

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Notebook References:

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unknown. The fact that 1 and the 6-chloro and 6-bromo analogues 19 and 21 were either inactive or had only slight activity in the wheat cylinder, pea segment, and pea curvature tests (James and Wain, 1968) suggests that these compounds do not possess auxin activity. Additional studies will be required to elucidate the mode of action of 6-methylanthranilic acid and analogues.

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Appendix H

Metribuzin Metabolites in Mammals and Liver Microsomal Oxidase Systems: Identification, Synthesis, and Reactions

Marian Saeman Bleeke and John E. Casida*

Mercapturic acid derivatives are the major urinary metabolites of metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] in intraperitoneally treated mice and orally treated rats, accounting for ~20% of the dose. These mercapturates of metribuzin and deaminometribuzin, in which the methylthio substituent is replaced by an *N*-acetylcysteinyl moiety, are also the major products on incubation of mouse and rat liver microsomes with metribuzin in the presence of *N*-acetylcysteine and NADPH. Other NADPH-dependent metabolites are deaminometribuzin and protein-bound material, the latter formed in large amounts only when *N*-acetylcysteine is not added. Deamination appears to be more important in rat than in mouse metabolism, both in vivo and in vitro. These findings suggest the formation of metribuzin sulfoxide and deaminometribuzin sulfoxide as activated intermediates. Oxidation of metribuzin and deaminometribuzin with *m*-chloroperbenzoic acid yields the corresponding sulfoxides, which react readily with *N*-acetylcysteine or protein in neutral aqueous solutions. The *N*-amino group is also cleaved on peracid oxidation, but *S*-methyl sulfoxidation occurs more rapidly.

Metribuzin or Sencor [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] is a potent photosystem II inhibitor used as a pre- and postemergence herbicide against a variety of broadleaf and grass weeds in potatoes, soybeans, and other tolerant crops (Draber et al., 1968; Eue, 1972). Acid hydrolyzes the SMe substituent from the metribuzin ring to give a diketo derivative (Frear et al., 1983a), and metal-catalyzed oxidation with *tert*-butyl hydroperoxide cleaves either the C–SMe or N–NH_2 group depending on the catalyst to form the diketo or deamino derivative, respectively (Nakayama et al., 1982). The metabolic fate of metribuzin is reported in several plant systems but not in animals. Soybean, sugarcane, and/or tomato form metribuzin *N*-glucoside and malonyl *N*-glucoside, the homoglutathione conjugate, and the deamino-, diketo-, and deaminodiketo derivatives (Hilton et al., 1974; Mangeot et al., 1979; Frear et al., 1983a,b). Metribuzin sulfoxide is a possible intermediate in metabolic formation of the homoglutathione conjugate (Frear et al., 1983b).

This investigation considers the metabolism of metribuzin in rats and mice and their liver microsomal oxidase systems. It also develops a chemical model for the observed reactions with emphasis on the importance of metribuzin sulfoxide as an activated intermediate.

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.

MATERIALS AND METHODS

Chromatography and Analysis. Thin-layer chromatography (TLC) utilized precoated silica gel 60 F-254 20

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Table I. Thin-Layer Chromatography R_f Values for Metribuzin and Related Compounds

unconjugated triazinones[a]	TLC R_f in indicated solvent system[b] CE	DEH
I-SMe	0.54	0.46
I-S(O)Me	0.09	
II-SMe	0.42	0.29
II-S(O)Me	0.04	
III-NH_2	0.23	0.10
III-H	0.32	0.20
IV-NH_2	0.60	0.59

conjugated triazinones[a,c]	TLC R_f in indicated solvent system[b] BAW	MBBW
I-SMA	0.50	0.69
I-SG	0.26	
II-SMA	0.50	0.69
II-SG	0.26	

[a] For compound designations, see Figure 1. [b] Silica gel chromatoplates developed with the following solvent systems: CE, chloroform–ethyl acetate, 1:1; DEH, dichloromethane–ether–hexane, 3:2:2; BAW, 1-butanol–acetic acid–water, 4:1:1; MBBW, methanol–benzene–1-butanol–water, 2:1:1:1. [c] R_f = 0.00 in CE and DEH.

× 20 cm chromatoplates (EM Laboratories, Inc., Elmsford, NY) with 0.25 and a 0.5 mm layer thickness for analysis and product isolation, respectively. Solvent systems and R_f values are given in Table I. Nonradioactive products were detected by ultraviolet (UV) quench and ^{14}C-labeled compounds by autoradiography. High-performance liquid chromatography (HPLC) was carried out on a μBondapak CN column (Waters Associates, Milford, MA). Radiocarbon content was quantitated by liquid scintillation counting (LSC) of liquid samples, radioactive gel regions scraped from TLC plates, and combusted solid samples. Melting points (mp) are uncorrected.

Proton nuclear magnetic resonance (^1H NMR) spectra were obtained with either a Perkin-Elmer R32B 90-MHz or a Bruker WM 300-MHz spectrometer. ^{13}C NMR spectra were recorded with the Bruker WM 300 instrument (75.4 MHz for ^{13}C). Samples were dissolved in deuterated solvents containing 1% tetramethylsilane as the internal standard. IR spectra were taken with a Perkin-Elmer 457 grating spectrophotometer and UV spectra with a Perkin-Elmer 576 spectrophotometer. Chemical ionization mass spectra (CI-MS) were recorded with a Finnigan 3200 instrument with methane as the reagent gas.

Chemicals. Figure 1 gives the structures and designations for metribuzin (I-SMe) and its metabolites and reaction products and related compounds. The chemicals are *N*-aminotriazinones (I), triazinones (II), triazinediones (III), thioxotriazinones, (IV), or various N-2-Me (V), N-4-Me (VI), or O-Me (VII) derivatives. Abbreviations for the ring substituents are given in Figure 1.

I-SMe (99% chemical purity) and [*carbonyl*-^{14}C]I-SMe (4.4 mCi/mmol; >99% radiochemical purity) were provided by Mobay Chemical Corp. (Kansas City, MO). Spectral data for I-SMe: ^1H NMR ($CDCl_3$) δ 4.94 (br s, 2 H, NH_2), 2.61 (s, 3 H, SMe), 1.43 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 161.0, 160.7, 151.0, 37.6, 27.5, 14.2; UV (MeOH) λ_{max} 227 nm (log ε 3.9), 293 (3.9).

Metabolism of [^{14}C]Metribuzin in Rats and Mice. [^{14}C]Metribuzin was administered orally to male albino rats (180–200 g) at 200 mg/kg and intraperitoneally (ip) to male Swiss-Webster mice (20–25 g) at 75 mg/kg, in each case with methoxytriglycol (MTG) as the carrier vehicle. The animals were held for 5 days in all-glass metabolism cages with ground rat chow and water ad libitum. Expired

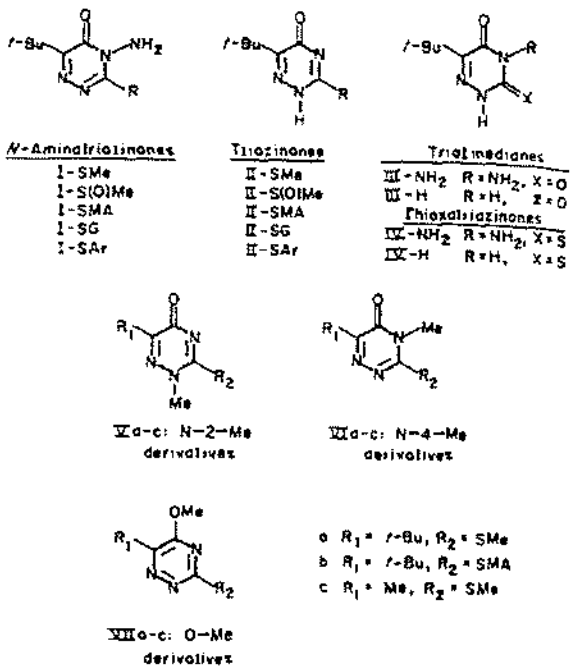

Figure 1. Structures and designations for metribuzin and related compounds. (Me = methyl; *t*-Bu = *tert*-butyl; Ar = 3,4-dichlorophenyl; SG = glutathionyl; SMA = *N*-acetylcysteinyl or mercapturic acid. The SMA derivatives Vb and VIIb are methylated).

$^{14}CO_2$ was monitored for the rats by collection in a monoethanolamine–methyl cellosolve, 2:1, mixture. The urine and a methanol extract of the feces were analyzed by LSC and TLC (CE and BAW). For isolation of the mercapturic acids, rat urine was diluted with an equal volume of saturated NaCl, acidified to pH 2 with 1 N HCl, and extracted with 3 volumes of ether–ethanol, 3:1, and the extract was subjected to preparative TLC (BAW) (mercapturic acids R_f = 0.50).

Metabolism of [^{14}C]Metribuzin in Rat and Mouse Liver Microsomal Enzyme Systems. Rats and mice as above were treated ip with phenobarbital at 80 mg/kg on each of 3 consecutive days with sacrifice on the fourth day. Livers from these phenobarbital-induced animals were homogenized at 20% (fresh weight/volume) in 0.1 M phosphate, pH 7.4, buffer. Centrifugal fractionation gave the soluble fraction (100000*g* supernatant) and the microsomal pellet (13000*g* supernatant and 100000*g* pellet washed once by resuspension and recentrifugation). These fractions were stored at −80 °C until used. Incubation mixtures in 0.1 M phosphate, pH 7.4, buffer (2.5 mL) contained the microsomal preparation (0.75 mL at 20% fresh liver weight equivalent) with one or more of the following additions: soluble fraction (0.50 mL), reduced nicotinamide adenine dinucleotide phosphate (NADPH) (2.2 mg), *N*-acetylcysteine (NAcCys) or glutathione (GSH) (5 mg), and [^{14}C]I-SMe (0.05 μCi, 20 nmol) added last in ethanol (20 μL). After incubation in a 25-mL Erlenmeyer flask with shaking for 2 h at 37 °C, the mixture was extracted with chloroform (3 × 2.5 mL). The aqueous phase was lyophilized to dryness and the residue extracted with methanol (3 × 2.5 mL). The dried ($MgSO_4$) chloroform fraction and the methanol fractions were concentrated under a stream of nitrogen and separated by TLC (CE, BAW). Methanol fractions from large-scale incubations (500 μg of I-SMe/flask) were subjected to TLC (BAW) for isolation of mercapturic acids. Soluene (Packard Instru-

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ment Co., Downers Grove, IL) was used to solubilize protein fractions for LSC. A comparative study employed enzyme from rats and mice that received no phenobarbital pretreatment.

Hydrolysis and Methylation of Mercapturic Acids. The TLC-purified mercapturic acid metabolites were subjected to acid hydrolysis (2 N HCl, 100 °C, 3 h), neutralized with NaOH, and extracted into chloroform for TLC analysis (CE). They were also methylated with diazomethane (a hazardous chemical; handle with caution) followed by separation by TLC (chloroform–ethyl acetate, 13:10; Vb R_f = 0.09, VIIb R_f = 0.21) and HPLC (hexane–ethyl acetate, 2:1; Vb elutes completely before VIIb) and analysis by CI-MS and UV.

Syntheses and Spectral Features of Triazinones, Triazinediones, Thioxotriazinones, and Related Compounds (Figure 1). The syntheses are based on procedures referenced in Neunhoeffer and Wiley (1978).

II-SMe. IV-H (3.5 g, 19 mmol, described below) was added to a solution of sodium (1.38 g, 60 mmol) in methanol (30 ml). Methyl iodide (1.3 mL, 21 mmol) was added, and the solution was heated at reflux for 15 min. Most of the methanol was then removed under vacuum, water (50 mL) was added, and the solution was neutralized with 2 N HCl. A precipitate formed, and the mixture was extracted with ethyl acetate. After drying ($MgSO_4$), the ethyl acetate was removed under vacuum. Recrystallization from chloroform–hexane yielded II-SMe (3.3 g, 87%, mp 199–200 °C): ^{1}H NMR (CD_3COCD_3) δ 2.55 (s, 3 H, Me), 1.33 (s, 9 H, *t*-Bu); ^{13}C NMR (CD_3COCD_3) δ 164.2, 160.0, 157.8, 37.1, 27.4, 12.4; IR (2% MeOH–$CHCl_3$) 1650 cm^{-1} (C=O); UV (MeOH) λ_{max} 236 (log ϵ 3.1); CI-MS *m/e* 200 (M + 1).

III-NH$_2$. I-SMe (200 mg, 0.93 mmol) was refluxed in 2 N HCl (50 mL) for 3 h. The solution was cooled, neutralized with 10% NaOH, and extracted with ether. Washing the ether layer with saturated NaCl, drying ($MgSO_4$), and solvent evaporation yielded III-NH_2 (145 mg, 79%, mp 167–168 °C): ^{1}H NMR ($CDCl_3$) δ 5.20 (br s, 2 H, NH_2), 1.33 (s, 9 H, *t*-Bu); ^{13}C NMR (CH_3COCH_3 + CD_3COCD_3) δ 152.8, 149.7, 148.1, 37.4, 27.8; UV (MeOH) λ_{max} 212 nm (log ϵ 3.7), 262 (3.7); CI-MS *m/e* 185 (M + 1).

III-H. II-SMe (200 mg, 1.0 mmol) was treated as above to obtain III-H (120 mg, 70%, sublimed 260–265 °C): ^{1}H NMR (CD_3COCD_3) δ 1.35 (s, t-Bu); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 155.9, 150.7, 149.9, 36.0, 26.7; UV (MeOH) λ_{max} 208 nm (log ϵ 3.7), 261 (3.7); CI-MS *m/e* 170 (M + 1).

IV-NH$_2$. Addition of trimethylpyruvic acid (from oxidation of pinacolone with alkaline $KMnO_4$; Saeman, 1984) (1.6 g, 12.3 mmol) to thiocarbohydrazide (1.3 g, 12.3 mmol; Aldrich) dissolved in refluxing water (20 mL) gave a fine white precipitate. The suspension was stirred for 1 h and filtered to yield IV-NH_2 (2.1 g, 84%, mp 219–220 °C): ^{1}H NMR (CD_3COCD_3) δ 6.43 (br s, 2 H, NH_2), 1.37 (s, 9 H, t-Bu); UV (MeOH) λ_{max} 204 nm (log ϵ 3.5), 270 (4.0); CI-MS *m/e* 201 (M + 1).

IV-H. Thiosemicarbazide (4.4 g, 48 mmol; Aldrich) in hot water (100 mL) was treated with trimethylpyruvic acid (6.3 g, 48 mmol) as above to obtain a white precipitate. NaOH pellets (3.8 g, 96 mmol) were added, the precipitate was dissolved, and the solution was heated for 30 min. After cooling and neutralization with 5 N HCl, the precipitate was filtered and recrystallized from ether to yield IV-H (3.9 g, 44%, sublimed 260–270 °C): CI-MS *m/e* 186 (M + 1).

Va, VIa, and VIIa. A solution of II-SMe (43 mg, 0.22 mmol) in methanol (5 mL) was treated dropwise with a diazomethane–ether solution until the bubbling stopped and the yellow color remained. The mixture was separated by preparative TLC (ether–hexane, 1:1) into three fractions with a combined yield of 32 mg (68%). Va: 8 mg (25% of products), R_f = 0.08; ^{1}H NMR ($CDCl_3$) δ 3.72 (s, 3 H, NMe), 2.55 (s, 3 H, SMe), 1.29 (s, 9 H, t-Bu); UV (MeOH) λ_{max} 236 nm (log ϵ 4.1); CI-MS *m/e* 214 (M + 1). VIa: 14 mg (44% of products), R_f = 0.22; ^{1}H NMR ($CDCl_3$) δ 3.45 (s, 3 H, NMe), 2.67 (s, 3 H, SMe), 1.37 (s, 9 H, t-Bu); UV (MeOH) λ_{max} 211 nm (log ϵ 4.0), 230 (3.8), 295 (3.9); CI-MS *m/e* 214 (M + 1). VIIa: 10 mg (31% of products), R_f = 0.47; ^{1}H NMR ($CDCl_3$) δ 4.03 (s, 3 H, OMe), 2.62 (s, 3 H, SMe), 1.37 (s, 9 H, t-Bu); UV (MeOH) λ_{max} 208 nm (log ϵ 3.9), 248 (4.1), 298 (3.6); CI-MS *m/e* 214 (M + 1).

Syntheses, Spectral Features, and Reactions of Metribuzin and Deaminometribuzin Sulfoxides [I-S(O)Me and II-S(O)Me] (Figure 1). *Peracid Oxidation of I-SMe.* A solution of [^{14}C]I-SMe (6 mg) in chloroform (0.5 mL) was treated with appropriate amounts of *m*-chloroperbenzoic acid (MCPBA) with stirring at 0 °C. Analyses involved TLC (CE) and LSC.

I-S(O)Me. A solution of I-SMe (150 mg, 0.70 mmol) in chloroform (6 mL) at 0 °C was treated with MCPBA (65 mg, 0.35 mmol) dissolved in chloroform (6 mL) and stirred for 5 min at 0 °C. The chloroform was removed under vacuum and the residue taken up in ether. Solvent evaporation and cooling on partial removal of the ether under vacuum with no applied heat precipitated I-S(O)Me (30 mg, 37% based on MCPBA): ^{1}H NMR ($CDCl_3$) δ 5.92 (br s, 2 H, NH_2), 3.25 [s, 3 H, S(O)Me], 1.48 (s, 9 H, t-Bu); ^{13}C NMR ($CDCl_3$) δ 166.9, 155.9, 149.8, 38.5, 37.2, 27.2. IR ($CHCl_3$) 1675 (C=O), 1050 cm^{-1} (S=O); CI-MS *m/e* 231 (M + 1).

II-S(O)Me. A solution of II-SMe (500 mg, 2.5 mmol) and MCPBA (700 mg, 3.8 mmol) in acetone (50 mL) at 0 °C was stirred for 2 h at 0 °C, the acetone was removed under vacuum, and the residue was taken up in ether. Partial removal of the ether gave II-S(O)Me as a precipitate (380 mg, 70%): ^{1}H NMR (CD_3COCD_3) δ 3.03 [s,, 3 H, S(O)Me], 1.34 (s, 9 H, t-Bu); ^{13}C NMR ($CDCl_3$) δ 164.6, 161.7, 159.3, 39.9, 37.7, 27.0; IR ($CHCl_3$) 1672 (C=O), 1050 cm^{-1} (S=O); CI-MS *m/e* 216 (M + 1).

I-SAr. 3,4-Dichlorobenzenethiol (6 μL, 0.05 mmol; Aldrich) was added to a solution of I-S(O)Me (10 mg, 0.043 mmol) in chloroform (0.5 mL). After 30 min at 25 °C, the reaction mixture was separated by TLC (0.5 mm silica gel, developed twice in ether–hexane, 1:1), and the band at R_f = 0.32 was scraped and extracted with ether to give I-SAr (9 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 7.82 (br s, 1 H, aromatic), 7.60 (br s, 2 H, aromatic), 4.98 (br s, 2 H, NH_2), 1.43 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 213 nm (log ϵ 4.4), 294 (4.0); CI-MS *m/e* 345 (M + 1).

II-SAr. 3,4-Dichlorobenzenethiol (60 μL, 0.48 mmol) was added to an acetone solution (6 mL) of II-S(O)Me, prepared in situ from II-SMe (65 mg, 0.33 mmol) and MCPBA (80 mg, 0.43 mmol) as described above. The solution was stirred overnight. II-SAr (78 mg, 70%, mp 220–223 °C) was obtained both as a white precipitate on partial removal of the acetone under vacuum and by preparative TLC of the supernatant (0.5 mm silica gel, acetonitrile–chloroform, 1:7, R_f 0.67, recovered by chloroform extraction): ^{1}H NMR (CD_3COCD_3) δ 7.89 (br s, 1 H, aromatic), 7.65 (br, d, 2 H, aromatic), 1.30 (s, 9 H, t-Bu); UV (MeOH) λ_{max} 234 (log ϵ 4.2); CI-MS *m/e* 330 (M + 1).

I-SMA. MCPBA (150 mg, 0.90 mmol) in acetone (5 mL) at 0 °C was added to I-SMe (300 mg, 1.4 mmol) in acetone (10 mL) at 0 °C. After 10 min this cold solution was added dropwise to NAcCys (500 mg, 3 mmol) dissolved in 0.1 M

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Table II. Metabolism of [^{14}C]Metribuzin by Phenobarbital-Induced Rat and Mouse Liver Microsomes in the Presence or Absence of *N*-Acetylcysteine and NADPH

^{14}C compd or ^{14}C fraction	radiocarbon recovery, %,[a] with indicated fortification[b]			
	none	NAcCys	NADPH	NAcCys + NADPH
Rat Liver Microsomes				
I-SMe	90	91	35	28
II-SMe	0.8	0.6	24	18
I-SMA + II-SMA[c] unidentified[d]	0	3	0	45
apolar	7	5	8	1
polar	1	0.4	19	7
bound	0.3	0.2	14	1
Mouse Liver Microsomes				
I-SMe	84	83	56	56
II-SMe	0.9	0.6	6	6
I-SMA + II-SMA[c] unidentified[d]	0	2	0	28
apolar	14	13	13	7
polar	0.6	0.8	8	3
bound	0.6	0.2	17	0.7

[a] Average of two independent studies with data for product yields varying by 0.8–1.2-fold between the experiments. [b] Mixtures of microsomes (150 mg fresh liver weight equivalent) with NAcCys (5 mg) and/or NADPH (2.2 mg) in 0.1 M phosphate, pH 7.4, buffer (2.5 mL) incubated for 2 h at 37 °C. [c] About equal amounts of I-SMA and II-SMA. [d] The unidentified metabolites are apolar (chloroform extractable), polar (methanol extractable), or protein bound. Apolar and polar unknowns include material at the origin or streaking on the plate. [e] >80% I-SMA with a small amount of II-SMA.

phosphate, pH 7.4, buffer (25 mL). After stirring 18 h at 25 °C, the solution was extracted with ether (2 × 20 mL), and the aqueous phase was then saturated with NaCl and extracted with ether–ethanol, 3:1 (3 × 40 mL), to recover crude I-SMA.

II-SMA. II-S(O)Me (100 mg, 0.47 mmol) was dissolved in acetone (5 mL) and treated as above to obtain crude II-SMA.

Derivative with BSA. A solution of [^{14}C]I-SMe (10 μg) and MCPBA (30–45 μg) was held in acetone (35 μL) for 30 min at 25 °C. After analysis for I-S(O)Me content by TLC (CE), the solution was diluted with acetone to 0.2 mL, added to BSA (10 mg) dissolved in 0.1 M phosphate, pH 7.4, buffer (2 mL), and stirred for several hours at 37 °C. An equal volume of 10% aqueous trichloroacetic acid was added. The precipitate recovered by centrifugation was redissolved in water (2 mL) and reprecipitated as above, and the procedure was repeated a third time to ensure that no soluble radioactivity remained. The precipitate was dissolved in Soluene for LSC.

RESULTS

Metabolism of [^{14}C]Metribuzin in Rats and Mice. The major urinary metabolite in rats is II-SMA (identification discussed below) and in mice chromatographs (BAW) in the position of I-SMA + II-SMA, in each species accounting for ~20% of the administered dose within 5 days after treatment. Although other urinary products are not identified, they are polar compounds, such as conjugates, and no one of them accounts for more than 5% of the dose. Rat feces contain 0.1–1% of the administered radiocarbon as unmetabolized I-SMe. The other rat fecal products are not identified but do not include any of the nonconjugated compounds shown in Figure 1 based on TLC cochromatography (CE, BAW) (Table I). No radioactivity is expired by rats as [^{14}C]carbon dioxide.

Table III. Triazinediones from Acid Hydrolysis of [^{14}C]Metribuzin and the [^{14}C]Mercapturic Acids Formed on in Vivo and in Vitro Metabolism in Rats and Mice

material hydrolyzed[a]	triazinedione, %[b]	
	III-NH_2	III-H
I-SMe	89	11
II-SMe	0[c]	100[c]
I-SMA + II-SMA[d]		
rat urine	0.5	99
rat enzyme	51	49
mouse enzyme	84	16

[a] 2 N HCl, 3 h, 100 °C. [b] Yields of III-NH_2 and III-H normalized to 100%. Additional products were not recovered on chloroform extraction or not resolved by TLC, respectively, as follows: I-SMe, 3 and 2%; rat urine, 5 and 0.5%; rat enzyme, 11 and 11%; mouse enzyme, 5 and 5%. [c] Results with unlabeled II-SMe based on TLC and product visualization by UV quench. [d] The percent radiocarbon recovery as mercapturic acids (I-SMA plus II-SMA) was ~20% in rat urine (Bleeke et al., 1984) and 45 and 28% in rat and mouse enzyme systems, respectively (Table II).

Metabolism of [^{14}C]Metribuzin in Rat and Mouse Liver Microsomal Enzyme Systems. Comparative studies revealed that phenobarbital induction increases NADPH-dependent metabolism of I-SMe by 2–3-fold in rats and about 50% in mice. [^{14}C]I-SMe undergoes relatively little metabolism or decomposition on incubation with phenobarbital-induced rat or mouse liver microsomes alone or fortified with NAcCys (Table II). Metabolism is greatly facilitated and new metabolites are formed on fortification with NADPH or a combination of NADPH and NAcCys. Deamination to II-SMe is more prominent with rat than with mouse liver preparations. Fortification with both NADPH and NAcCys gives a mixture of I-SMA and II-SMA, as discussed later. Protein-bound ^{14}C derivatives are major NADPH-dependent products in the absence of NAcCys but not in its presence, suggesting that the activated intermediates forming the I-SMA plus II-SMA mixture with NAcCys are also trapped by reaction with protein. TLC analysis (CE and BAW, Table I) revealed that the apolar and polar unknowns do not include I-S(O)Me, I-SG, II-S(O)Me, II-SG, and IV-NH_2 and that little (<1%) III-H is present. III-NH_2 is occasionally detected as a minor (1–2%) NADPH-dependent metabolite formed in the absence of added thiols.

A GSH conjugate appears to form as a microsomal metabolite under suitable conditions. Thus, a product of appropriate TLC characteristics (BAW) is obtained with suitable mouse liver enzyme systems in yields as follows: microsome plus NADPH <1%; soluble fraction plus NADPH 2%; microsome plus soluble fraction plus NADPH 16%; microsome GSH plus NADPH 13%.

Identification of Mercapturic Acids in Urine and Enzyme Systems. I-SMA and II-SMA are not separated in the TLC systems examined (Table I). They were therefore identified as mercapturates by TLC cochromatography and as I-SMA or II-SMA by degradation and spectroscopic methods described below.

Acid hydrolysis of I-SMe and II-SMe gives primarily III-NH_2 and III-H, respectively (Table III). Analogous reactions of the mercapturic acids serve to distinguish I-SMA from II-SMA. The rat urinary mercapturic acid fraction yields almost only III-H, strongly indicating that it is almost entirely II-SMA (Table III). The mouse mercapturic acids were not isolated for identification, but direct acid hydrolysis of the urine yields 35% III-NH_2, 5% III-H, and 60% polar products. The isolated mercapturic acids from the mouse enzyme give III-NH_2 and III-H in a 5:1 ratio, approximating the 8:1 ratio for hydrolysis of [^{14}C]I-SMe, but in marked contrast to the 1:1 ratio for the

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Table IV. Comparison of Dimethylated Mercapturic Acids (Vb and VIIb) with Related *S*-Methyl Derivatives

methylated deriv[a]			NMR, δ				UV, λ_{max},
compd	R_1	R_2	S-Me	N-2-Me	N-4-Me	O-Me	nm
N-2-Me Derivatives							
Va	t-Bu	SMe	2.55	3.72			236
Vb	t-Bu	SMA	*b*	*b*			239
Vc	Me	SMe	2.48	3.66			236
N-4-Me Derivatives							
VIa	t-Bu	SMe	2.67		3.45		211, 230, 295
VIc	Me	SMe	2.60		3.35		231, 284, 295
O-Me Derivatives							
VIIa	t-Bu	SMe	2.62			4.03	208, 248, 298
VIIb	t-Bu	SMA	*b*			*b*	211, 246, 294
VIIc	Me	SMe	2.58			4.02	247, 305

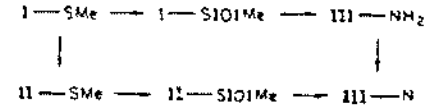
[a] Series a from synthesis, b from methylation of a rat urinary metabolite, and c from the literature (Daunis et al., 1971). [b] Not determined.

rat enzyme products (Table III). These findings suggest that the mercapturic acid fraction is >80% I-SMA with a small amount of II-SMA for the mouse but about equal amounts of I-SMA and II-SMA for the rat. Thus, the ratio of III-H to III-NH_2 both in vivo and in vitro suggests that deamination of I-SMe is more important in rats than in mice.

The UV spectra of triazinones give absorbances characteristic of the substitution pattern on the ring, allowing comparison of the mercapturic acid metabolites with I-SMe and II-SMe. The TLC-isolated mouse microsomal product is primarily I-SMA since the spectrum in methanol has two main absorbances [λ_{max} as nm (log ε)], i.e., 223 (4.0) and 296 (3.6), very similar to those of I-SMe with 227 (3.9) and 293 (3.9). The rat urinary mercapturic acid fraction is almost only II-SMA, giving 238 (4.2), much like that of II-SMe with 236 (3.1).

Methylation of the rat urinary mercapturic acid fraction gives two dimethyl derivatives with spectral features further supporting the identification of this metabolite as primarily II-SMA. Thus, treatment with diazomethane, followed by HPLC and TLC, results in the isolation of two products. The CI-MS for each compound gives a $(M + 1)^+$ signal of 343, the expected mass of II-SMA after methylation of both the ring and the carboxylic acid. Table IV compares the spectral data of the two methylated derivatives of the mercapturic acid (Vb and VIIb) with the three methylated products from II-SMe, V–VIIa, and the related compounds V–VIIc. The UV λ_{max} values of the methylated mercapturic acid derivatives correspond to those of the N-2-Me (239 nm) and the O-Me (246 nm) compounds. Clearly, the mercepturic acid metabolite is methylated at the N-2 and O positions as anticipated for II-SMA.

Peracid Oxidation of Metribuzin and Deaminometribuzin. The reaction of I-SMe with MCPBA in chloroform at 0 °C proceeds as follows (Figure 2):



I-S(O)Me is the principal product formed with 1 part of MCPBA to 2 parts af I-SMe (30–40% yield based on MCPBA). With increasing oxidant (1:1, 2:1, and 4:1

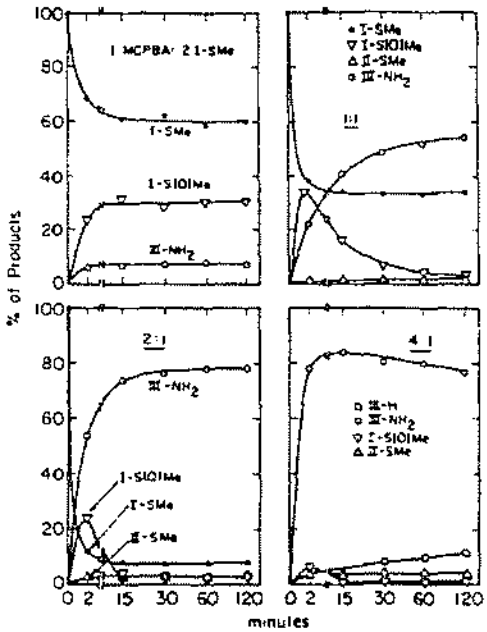


Figure 2. Reaction rates and product profiles for varying ratios of metribuzin to *m*-chloroperbenzoic acid in chloroform at 0 °C.

MCPBA–I-SMe), I-S(O)Me is rapidly converted to III-NH_2, which becomes the major final product. At the 4:1 ratio very little I-S(O)Me is detected, even at short reaction times. The product profile indicates that I-S(O)Me serves as the intermediate in forming III-NH_2, a proposal confirmed by finding that MCPBA directly converts I-S(O)Me to III-NH_2.

The deamino derivatives II-SMe and III-H are minor products. I-SMe is oxidatively deaminated to II-SMe but at a very slow rate compared with sulfoxidation. III-H is the final product resulting from oxidation at both the SCH_3 and NH_2 groups and becomes significant only with a large excess of MCPBA. Studies comparable to those shown in Figure 2 but with II-SMe establish a much slower conversion rate for II-SMe to II-S(O)Me and in turn to III-H than for the analogous reactions in the I-SMe series. II-S(O)Me is formed almost quantitatively on oxidation of II-SMe with a slight excess of MCPBA, showing that in this case sulfoxidation is much faster than subsequent cleavage to the diketo compound. II-S(O)Me is not detected as a product on treatment of I-SMe with MCPBA because little II-SMe is formed and its subsequent oxidation is slow.

Preparation, Properties, and Reactions of Metribuzin and Deaminometribuzin Sulfoxides. I-S(O)Me and II-S(O)Me are formed in 70–80% yields (NMR) based on MCPBA and can be isolated in 37 and 70% yields, respectively, on MCPBA oxidation of I-SMe in chloroform and of II-SMe in acetone at 0 °C. I-S(O)Me is rarely obtained in >80% purity due to decomposition to III-NH_2; partial decomposition occurs within 1 day either neat or in chloroform at 0 °C and complete breakdown is evident within 8 h at 25 °C. II-S(O)Me, obtained in >95% purity, is stable for weeks as a crystalline solid at 0 °C and for at least a few days at 25 °C.

Sulfoxides I-S(O)Me and II-S(O)Me are identified on the basis of their reactions, described below, and of their spectral features that are characteristic of sulfoxides (Silverstein et al., 1981). They each give CI-MS base peaks appropriate for monooxygenated derivatives, a strong IR band at 1050 cm^{-1} associated with the S=O absorption, a ^1H NMR spectrum with a signal for the S(O)Me protons

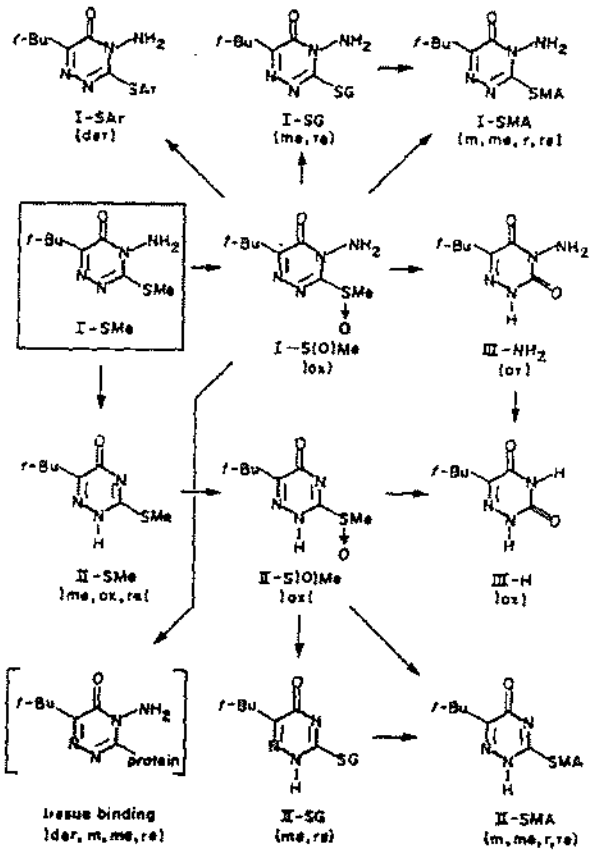


Figure 3. Reactions of metribuzin. Abbreviations: der, derivatization; m, mouse; me, mouse enzyme; ox, MCPBA; r, rat; re, rat enzyme.

shifted 0.5–0.6 ppm downfield from the SMe protons of the parent compound, and a ^{13}C signal for the S(O)Me substituent shifted 24–27 ppm downfield with respect to the parent SMe group.

The thiol 3,4-dichlorobenzenethiol reacts quickly with I-S(O)Me and II-S(O)Me, displacing the S(O)Me group to form thioethers I-SAr and II-SAr, respectively, which are crystalline or solid products; an analogous reaction was used to characterize cyanatryn sulfoxide (Bedford et al., 1975). I-S(O)Me and II-S(O)Me also react with NAcCys to give products with chromatographic properties identical with those of the mercapturate metabolites of I-SMe discussed above.

The protein BSA also reacts readily with I-S(O)Me at physiological pH. Thus, addition of the crude reaction mixture of [^{14}C]I-SMe and MCPBA (containing ~25% I-S(O)Me) to BSA leads to binding of about 5% of the total radioactivity compared to only 0.1% binding for an equivalent reaction of BSA and [^{14}C]I-SMe with no oxidant.

DISCUSSION

Several chemical and metabolic reactions of I-SMe are given in Figure 3. Sulfoxides I-S(O)Me and II-S(O)Me, from MCPBA oxidations of I-SMe and II-SMe, respectively, are reactive, electrophilic compounds. I-S(O)Me is harder to isolate and less stable than II-S(O)Me. Further oxidation of I-S(O)Me with MCPBA gives diketo derivative III-NH_2. The mechanism of this conversion (formally a replacement of S(O)Me with OH followed by tautomerization) is not known. Although a possible short-lived intermediate, no sulfone has been isolated or identified by TLC or NMR of the reaction mixture. A side reaction in MCPBA oxidation of I-SMe is the loss of NH_2, resulting in II-SMe. Reactions at both sites to give III-H is important only with a large excess of MCPBA. No intermediates are detected in the deamination reaction.

In vitro metabolism of I-SMe with rat or mouse liver microsomal enzymes occurs at both the N-NH_2 and SMe substituents. The enzymes involved require NADPH and are induced by phenobarbital, suggesting an oxidative mechanism for both processes. In contrast, deamination of I-SMe in plants appears to be a reductive cleavage (Fedtke and Schmidt, 1983). I-S(O)Me is the proposed intermediate in yielding thiol conjugates and protein derivatives. Analogous pathways are known in mammalian metabolism of other sulfur-containing pesticides (Casida et al., 1975; Bedford et al., 1975; Hubbell and Casida, 1977; Crawford et al., 1980; Hutson, 1981). Formation of I-SG requires oxidative activation by microsomes and is not dependent on the soluble fraction, indicating that conjugate formation is mediated by a microsomal GSH S-transferase or is due to direct chemical reaction between I-S(O)Me and GSH; the latter proposal is consistent with the demonstrated reactivity of chemically formed I-S(O)Me. Systems lacking thiols generally give more unidentified products and streaking on TLC, possibly due to decomposition of I-S(O)Me to III-NH_2 and other compounds. No IV-NH_2 is detected in the microsomal oxidations, suggesting that I-SMe does not undergo S-demethylation. No attempt was made to look for products resulting from oxidation of the *tert*-butyl group.

The findings on in vivo mammalian metabolism parallel the in vitro results. Deamination appears more important in rats than in mice, as is also the case for microsomal metabolism. The lack of [^{14}C]carbon dioxide formation by rats treated with [*carbonyl*-^{14}C]I-SMe suggests that ring opening followed by decarboxylation does not occur. Mercapturic acid formation is a major metabolic pathway, consistent with initial formation of a sulfoxide followed by conjugation with GSH. Sulfoxidation in mice appears to activate I-SMe for conjugation with GSH until the thiol is depleted and then for reaction with tissue proteins and associated hepatotoxicity (Bleeke et al., 1984).

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Luis Ruzo and Ian Holden of this laboratory assisted in the MS and NMR analyses, respectively.

Registry No. I-SMe, 21087-64-9; I-S(O)Me, 90269-30-0; I-SAr, 90269-27-5; I-SMA, 90269-25-3; II-SAr, 90269-28-6; II-SMe, 35045-02-4; II-S(O)Me, 90269-26-4; II-SMA, 90269-24-2; II-H, 52236-30-3; III-NH_2, 56507-37-0; IV-H, 66392-60-7; IV-NH_2, 33509-43-2; Va, 79988-50-4; VIa, 62036-60-6; VIIa, 90269-29-7; trimethylpyruvic acid, 815-17-8; thiocarbohydrazide, 2231-57-4; thiosemicarbazide, 79-19-6; 3,4-dichlorobenzenethiol, 5858-17-3.

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Interaction between γ-Hexachlorocyclohexane and the Gastrointestinal Microflora and Their Effect on the Absorption, Biotransformation, and Excretion of Parathion by the Rat

Robert W. Chadwick,* M. Frank Copeland, Ritchie Froehlich, Nathaniel Cooke, and Douglas A. Whitehouse

Pretreatment of rats with the organochlorine insecticide lindane reduced the estimated absorption rate of parathion from the gastrointestinal tract. Lindane pretreatment also significantly reduced the metabolism of parathion to *p*-nitrophenol in vivo. Lindane pretreatment altered the gastrointestinal (GI) microflora by increasing the ratio of anaerobes to aerobes. Consistent with this alteration was a significantly greater retention of unaltered parathion and the microbial metabolite aminoparathion in the GI tract of the lindane-pretreated rats 1 h after the administration of parathion. Enhanced conversion of parathion to aminoparathion together with a slower absorption rate may play a role in the antagonism parathion toxicity by lindane.

In the past it has been widely reported that pretreatment of animals with halogenated chemicals antagonizes the toxicity of organophosphate pesticides (Ball et al., 1954; Triolo and Coon, 1966; Townsend and Carlson, 1981; Iverson, 1976; Mendoza and Shields, 1976; Welch and Coon, 1964; Triolo et al., 1970). Moreover, this antagonism has generally been attributed to induction of either esterases or hepatic mixed function oxidases. While examining the dynamics of the absorption, biotransformation, and excretion of parathion [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate], we observed that pretreatment of rats with lindane (γ-hexachlorocyclohexane) impaired the estimated absorption rate of parathion. Corresponding to this impaired absorption, significantly lower excretion rates during the initial absorption were also observed in the lindane-pretreated rats. A series of experiments designed to determine the mechanism by which lindane elicited these effects produced data that indicate that altered gastrointestinal microflora may contribute to the protective effect of lindane and possibly other halogenated chemicals against the toxicity of ingested organophosphate pesticides.

MATERIALS AND METHODS

Apparatus. Gas–liquid chromatographic analysis was performed on a Tracor Model MT-220 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) and a flame photometric detector (FPD). Urinary *p*-nitrophenol was determined by ECD on 1% SP1240-DA on 100–120-mesh Supelcoport at 170 °C with 60 cm^3/min of 95:5 methane–argon carrier gas. Derivatized diethyl phosphorothioic acid (DETP), diethylphosphoric acid (DEP), and paraoxon [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphate] were determined by FPD with 3% OV-1 on 80–100-mesh Chromosorb W at 210 °C. Aminoparathion and parathion were analyzed by FPD with 3% QF-1 on 80–100-mesh Gas-Chrom Q at 185 °C. Air, H_2, and N_2 carrier gas flows were regulated at 100, 50, and 60 cm^3/min, respectively.

Reagents. [*ring*-2,6-^{14}C]Parathion (specific activity 12.2 mCi/mmol and 98% purity) was obtained from Amersham Corp., Arlington Heights, IL. Parathion, aminoparathion, DETP, DEP, paraoxon, and lindane were obtained from the EPA, Health Effects Research Laboratory Analytical Reference Standards Repository, Research Triangle Park, NC. Pentafluorobenzyl bromide (PFB-Br) was obtained from Aldrich Chemical Co., Milwaukee, WI. Tetrahexylammonium hydrogen sulfate was obtained from Regis Chemical Co., Morton Grove, IL; 3% QF-1 on 80–100-mesh Gas-Chrom Q and 3% OV-1 on 80–100-mesh Chromosorb W were obtained from Applied Science Laboratories, Inc., State College, PA. 1% SP-1240 DA on 100–120-mesh Supelcoport was obtained from Supelco Inc., Bellefonte, PA. Thioglycollate medium was obtained from Becton, Dickinson and Co., Cockeysville, MD. Bacto nutrient broth was obtained from Difco Laboratories, Detroit, MI.

Procedures. Separate experiments were conducted to determine (1) the effects of pretreatment with lindane on

U.S. Environmental Protection Agency, Health Effects Research Laboratory, Developmental Biology Division (MD 67), Perinatal Toxicology Branch, Research Triangle Park, North Carolina 27711.

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Data Review:

Study Identification:

Study Title: The Metabolism and Excretion of SENCOR in Rats.

EPA Identification Numbers:

Sponsor: Mobay Chemical Corporation
Chemagro Agricultural Division
Kansas City, Missouri 64120

Testing Laboratory: Chemagro Division of Baychem Corporation
Research and Development Department

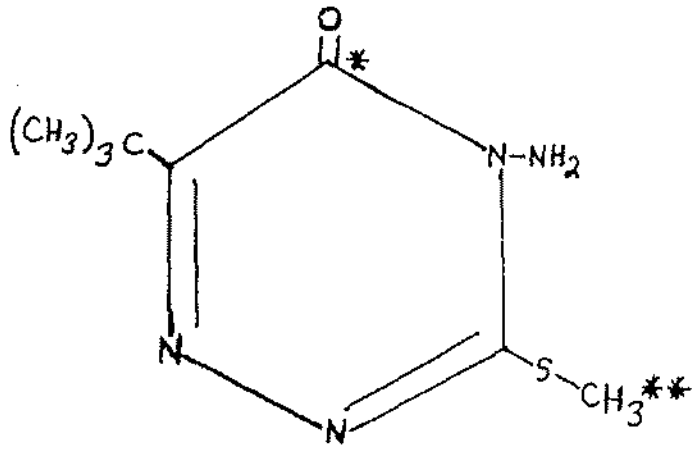
Report Number: 33366

Date of Study: May 1, 1972
Revised July 5, 1973 (to add additional information)

Study Authors: D.R. Flint
R.R. Gronberg
F.E. Sandie

Study Director: T.B. Waggoner

Test Compound: SENCOR [4-Amino-6-t-butyl-3-(methylthio)-1-2,4-triazin-5(4H)-one] (below) initially labeled with carbon-14 in the carbonyl group* and with tritium in the s-methyl group**.



Radiolabelled SENCOR

Dosages: First excretion study: 4 mg SENCOR - ^{14}C, ^{3}H in 0.8 ml 50% aqueous ethanol for a dose rate of 20 mg/kg in a 200 gm rat given orally by gavage (stomach tube).

Second excretion study and tissue residue studies: SENCOR (presumably ^{14}C labelled only , although not clearly stated) administered orally as a suspension in 0.5% aqueous gum tragacanth For animals weighing 150 to 165 gm, dosage volumes of 0.75 to 1.00 ml per animal were administered (presumably by gavage). The dose rates were calculated as 100 and 50 mg/kg for these studies.

Test Animals: Rats, Sprague-Dawley strain
Sprague-Dawley Company

Experimental: A copy of the experimental section from the investigators report is appended.

There was no clear indication as to how many animals were used at study initiation, however the results section states that one male and one female rat were used for the first excretion study, 2 males for the second excretion study and from the tables, 2 males and 2 females for the tissue residue studies. Also the age and sex of the animals was not given (although the results sections mentions male and female).

Apparently 2 excretion studies were conducted, one using glass metabolism cages with collection of respiratory gases and the other study using plastic with no collection of gases.

There was no mention of the purity of the test compound (a statement was made: "All equipment was standard except as listed and all chemicals were reagent grade or better.").

There was no indication of the time period for observation of animals (Guidelines state 7 days or until 90+% of the administered dose is excreted, with the animals in individual metabolism cages), although the individual tables in the final report state collection times.

The investigators examined expired air (only in the initial study for both ^{3}H and $^{14}CO_2$), urine, feces, blood, plasma, liver, kidney, heart, brain, muscle, testes, ovaries and fat. There was apparently no analysis of bone, lungs, spleen or residual carcass.

Results:

Excretion Studies:

The first study (using ^{14}C, ^{3}H labelled SENCOR) involved only 2 animals, one male and one female. The investigators reported sex related differences in excretion where in the male, 60.7% of the recovered radioactivity was found in the feces and in the female, 57.4% of the recovered radioactivity was found in the urine (over 90% of ^{14}C was recovered in urine and feces of both animals over a 16 day period). These values probably include measured ^{3}H levels as the total values on Table I do not totally agree. They further stated that no ^{14}C was recovered in the expired air. Sex related differences were also seen in the blood and tissue studies (to be discussed later).

The second study used 2 male rats (using only ^{14}C labelled SENCOR). The investigators found 45.89% of the radioactivity in the feces and 56.27% in the urine, from these finding they justified their reason for not collecting expired air, since the total was 102.16% of the administered radioactivity. See Table I. The excretion peak levels from this study were generally in agreement with the earlier study.

Table I: Excretion of Radioactivity (% of administered radioactivity)

Hours Post-Administration	Male		Female		2 Males	
	Urine	Feces	Urine	Feces	Urine	Feces
6.0	-	-	-	-	7.86	-
7.0	8.21	0.08	7.27	0.06	-	-
7.5	-	-	-	-	-	0.75
9.0	-	-	-	-	3.24	-
12.0	4.78	9.21	10.23	0.93	5.09	-
18.0	13.51	4.77	17.70	9.08	-	-
24.0	4.59	6.43	8.50	6.73	18.90	-
30.0	2.42	25.23	2.85	12.73	-	29.80
48.0	1.25	6.38	3.02	7.04	9.52	21.76
72.0	0.52	2.60	1.10	1.78	0.45	2.74
96.0	-	-	-	-	0.47	0.36
100.0	0.26	0.39	0.41	0.05	-	-
120.0	-	-	-	-	0.36	0.87
124.0	0.11	0.10	0.20	0.05	-	-
16 days	0.36	0.34	0.57	0.14	-	-
TOTAL	36.00	55.53	51.85	38.59	45.89	56.27

Data extracted from CHEMAGRO Report No. 33366 Tables I and I1.

Tissue Residue Studies:

These determinations reportedly involved 2 male and 2 female rats. The investigators stated that the residue levels were "generally similar" between the male and female rats except at 28 hour (after administration) interval which showed the females retaining more of the radioactivity in all tissues examined. After this time point the decline is similar, however the females still show slightly higher levels. There were not enough animals for statistical evaluation. The investigators further state that this was due to "sex-related differences in rates of absorption, metabolism, distribution and/or excretion". The table which they present for "half-lives" compares different interval measurements. See Table II.

Table II: Radioactive Residues in Rat Tissues (estimated "half-lives" of total ^{14}C in hours)

Tissue	Male†	Female††
Brain	21.1	22.4
Heart	26.4	33.6
Liver	30.4	33.6
Kidney	26.9	31.2
Muscle	21.3	24.5
Testes or Ovaries	18.4	30.4
Fat	25.0†††	24.8
Blood Plasma	19.1	27.2

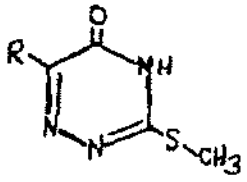
† - determined over a 24 to 96 hour interval.
†† - determined over a 48 to 96 hour interval.
††† - apparent biphasic decay curve after 24 hours.

Data extracted from CHEMAGRO Report No. 33366 Table III.

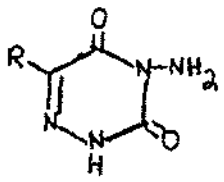
The investigators noted high tissue residue levels in liver and kidney (stated "presumably due to concentration in these organs for detoxification and elimination"). As can be seen in Table II the female rat presented with higher residue levels in heart, kidney, sex organs and blood plasma than the male rat.

Metabolite Identification:

From earlier metabolism studies in the soybean plant, three metabolites have been identified.



A



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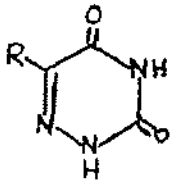


C

R = tertiary butyl

A - DA, deaminated SENCOR, also called BAY Dic 2058
B - DK, diketo SENCOR
C - DADK, deaminated diketo SENCOR, also called BAY Dic 2164

These metabolites were also identified in the animal studies. However, not all the residues were accounted for in the present study and many of the methods employed by the investigators destroyed much of the primary metabolites; this was especially true for the conjugate hydrolysis methods. The investigators should have employed non-harsh methods which could have involved the pre-separation of the metabolites prior to analysis and then study each metabolite separately.

Urine:

The investigators employed thin-layer chromotography (TLC) methods for urine studies. They observed that very polar solvent systems were needed to separate the samples and stated that this indicated that there were "either highly polar metabolites or, more likely, conjugated metabolites".

Enzyme incubation did not substantially change the pattern urine metabolites. The investigators then employed acid hydrolysis and found that one third of the radioactivity in the urine was rendered organoextractable. The organoextractable fraction was submitted to gas chromotographic analysis and SENCOR, DA, DK and DADK metabolites were found.

From other experiments the investigators stated that they found that the conditions of hydrolysis (not given) can affect a near complete de-thiomethylation of SENCOR and the DA metabolite to produce the DK and DAOK metabolites, therefore the procedure of using acid hydrolysis after enzyme treatment was not an accurate determination of urine metabolic distribution.

Studies with potatoes found that incubation in buffers of near neutral pH at 37°C could release significant amounts of SENCOR without other treatment.

A pooled 24 hour rat urine specimen was first deproteinized with perchloric acid and then extracted twice with isopropyl ether (IPE). The IPE extracts were analyzed by gas chromatography revealing small amounts of SENCOR and the 3 metabolites. The water soluble portion was analyzed by gel filtration. Two large fractions were found and were further treated by hydrolysis and ion-exchange chromatography. Although the investigators state that work in the area is not complete, they feel that significant amounts of the fractions they found from gel filtration are conjugates of SENCOR and its metabolites.

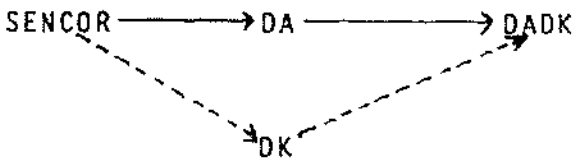
Tissues:

Liver and muscle tissues from male and female rats were homogenized in a two-phase water-chloroform system and each phase was assayed. They found slight differences in liver distribution of the compound between the male and female rats, whereas muscle distribution showed no sex related differences. The insoluble residue from the 28 hour female rat liver tissue extraction was also assayed using various enzymatic and hydrolytic methods (below).

The investigators evaluated several procedures and decided to employ a pepsin digestion followed by an acid hydrolysis of the aqueous phase. They were able to render 94-96% of the activity in the muscle, 55-78% of the activity in the liver and 43-58% of the activity in the kidney organosoluble. They could also render 63-96% of the activity of the brain and heart organosoluble without acid hydrolysis.

Tissues from male and female rats, collected at 4 and 28 hours after ^{14}C-SENCOR administration showed similar patterns of metabolites (no sex related differences). They determined that the DA metabolite appears early with the DK and DADK metabolite being produced at later intervals.

The scheme is as follows:



The investigators state that "the solid line indicates the more active pathway". However it is noted that on page 15 of the report both dotted lines point to "DK" and on page 16 one dotted line points to "DK" and the other to "DADK". The latter is consistent with the findings of the report.

Feces:

In a preliminary investigation the investigators tried organic extraction with acetone, methanol and then water. They were unable to extract the isotopes. TLC analysis yielded little information. No other procedures were tried.

Conclusions:

The excretion studies found sex related differences with the males excreting the radiolabel primarily in the feces and the females excreting the label primarily in the urine, however this reviewer feels that an inadequate number of animals was used in this study (one male and one female in one study and two males in another study). Tissue distribution studies also suggested slight sex related differences in distribution up to the 28 hour interval (after administration) with similar patterns of reduction in residue levels after that time point (however the females tended to present with higher overall levels at all time points measured). These studies also used an inadequate number of animals.

The investigators found a metabolic scheme for SENCOR in rats that was similar to what was found in an earlier study in soybeans. The metabolites that were identified are:

deaminated SENCOR (DA), also called BAY Dic 2058
diketo SENCOR (DK)
deaminated diketo SENCOR (DADK), also called BAY Dic 2164

Additional metabolites were not identified.

The following are the study deficiencies:

1. The numbers of animals used was inadequate.
2. The age of the animals was not provided.
3. The purity and clear isotope identification of the test compound was not given.
4. Rationale for time frame used for collection of urine, feces and expired air since there should have been some time points earlier than the 7.0 hour in one study and 6.5 in the other.
5. There was no tissue analysis of bone, lungs, spleen and residual carcass.

Core Classification: Core-Supplementary Data based on above deficiencies.

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The Metabolism and Excretion of SENCOR in Rats

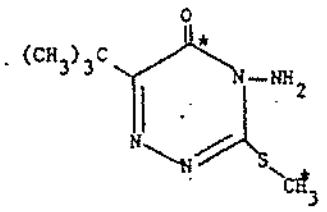
INTRODUCTION

SENCOR (BAY 94337) is a pre- and post-emergence herbicide being developed under license from Farbenfabriken Bayer AG for use with soybeans, potatoes and other crops for the control of broadleaf and grass weeds; it has also shown good potential as an aquatic herbicide. Possible residues in food crops require knowledge of their degradation and elimination in animal systems. The following study describes the metabolism and excretion of SENCOR in rats.

EXPERIMENTAL

Materials

SENCOR (I) [4-Amino-6-*t*-buryl-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] was synthesized by Koch *et al.* (1) labeled with carbon-14 in the carbonyl group and with tritium in the S-methyl group. The radiocarbon-labeled material used in later studies was synthesized by Robinson (2). The structure and positions of the labels are shown below:



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SENCOR metabolites, identified in a previous study (3), were obtained from Farbenfabriken Bayer AG, West Germany. The three metabolite standards, whose structures are shown below, were identified as follows:

II = DA, deaminated SENCOR (BAY Dic 2058)

III = DK, diketo SENCOR

IV = DA,K, deaminated diketo SENCOR (BAY Dic 2164)

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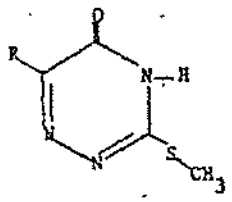
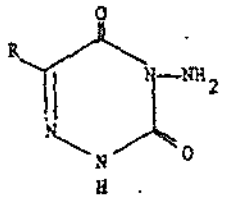
EXPERIMENTAL (Continued)

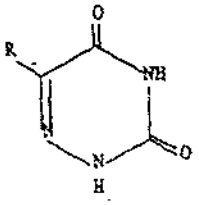
Rats used in this scudy were albino laboratory rats obtained from Sprague-Dawley Company. The animals were maintained on Purina Laboratory Chow and tap water *ad libitum* before and during all experiments. The animals were allowed to become accustomed to local surroundings for at least three days before beginning experimentacion.



II



III



IV

R = tertiary bucyl

All equipmenc was standard except as listed and all chemicals were reagent grade or better.

Methods

Dose Preparation and Administration. Animals used in the first excretion study were given SENCOR-^{14}C,^{3}H in 50% aqueous ethanol. The technical macerials were dissolved in the vehicle and given to each animal via stomach tube. The animals weighed ca. 200 grams each and each was given 4 mg SENCOR in 0.8 ml vehicle for a dose rate of 20 mg/kg.

Animals used in the second excretion study and in the tisswe residue study were also given SENCOR orally. Larger dose rates were employed in these studies, however, and complete solution was not possible in a small volume of any desirable solvent. SENCOR was administered to these animals as a suspension in 0.5% aqueous gum tragacanth. Animal weights were 150-165 grams and dose volumes were 0.75 - 1.00 ml per animal. The dose rates were 100 and 50 mg/kg for the second excretion and tissue residue studies, respectively.

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EXPERIMENTAL (Continued)

Sample Collection and Storage. In the first excretion study, urine, feces and expired gases were collected by means of Roth all-glass metabolism cages. Expired gases were collected in ethanolamine towers attached to the cages. Air was first passed through Drierite/Ascarite columns, then through the cages and finally through the adsorption towers at a rate of about 300 cc/min. Aliquots of the ethanolamine solution were collected periodically for radioassay.

Urine and feces were collected in the second excretion study using plastic metabolism cages (Maryland Plastics, Incorporated). The plastic cages were equipped to separate urine and feces but had no facility for the collection of expired gases. The cages were modified to permit immersion of the urine collection vessel in an ice-bath.

For tissue sample collection, the animals were sacrificed at appropriate intervals by decapitation under diethyl ether anesthesia. Blood was immediately drained from the carcass into 5 ml 0.85% saline containing 10 USP units of ammonium heparin/ml. The blood mixture was sampled immediately for radioassay then centrifuged and the plasma sampled for radioassay. The remaining sample was stored frozen. Tissues were collected immediately after exsanguination. They were weighed and immediately frozen for storage.

Urine was sampled upon collection for radioassay and the remainder stored frozen. Feces were frozen immediately upon collection.

Radiometric Analyses. Quantitative analysis of all samples was accomplished by radiometric assay using liquid scintillation spectrometry. Samples were measured in a Tri-Carb Liquid Scintillation Spectrometer (Model 3375, Packard Instrument Company) equipped with automatic external standardization (AES) for the determination of absolute activity. Various samples were prepared for radiometric analyses as follows:

1. Whole blood (diluted with saline) was applied directly to approximately 100-200 mg absorbent cotton in aliquots of 0.1-0.5 ml and carefully dried under heat lamps. The cotton and blood were then combusted in a Tri-Carb Oxidizer (Model 305, Packard Instrument Company) according to the manufacturer's instructions.

2. Blood plasma (obtained from centrifugation of the diluted whole blood) was pipetted directly into the sample-oxidizer cocktail in aliquots of 0.1 to 1.0 ml for direct assay. The scintillation cocktail consisted of 4 ml ethanolamine, 9 ml methanol and 7 ml scintillator solution containing 15 gm PPO (2,5-diphenyloxazole) and 1 gm bis MSB [p-bis-(o-methylstyryl)-benzene] per liter of toluene.

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EXPERIMENTAL (Continued)

3. Urine was assayed directly in amounts of 0.05 to 1.0 ml in a "Triton-X" scintillation cocktail composed as follows: 5 ml aqueous sample (diluted to 5 ml with water if necessary), 5 ml Triton X-100 and 10 ml scintillator solution containing 42 ml Spectrafluor PPO-POPOP (Amersham/Searle, Incorporated) per liter of toluene.

4. Feces were ground in the frozen state by first cooling the frozen fecal pellets contained in stainless steel cup in a dry ice/acetone bath. The cup was then fitted to an Omni-mixer (Ivan Sorvall, Incorporated) for grinding at high speed. Approximately 200 mg of the ground, frozen feces were weighed accurately onto cotton for combustion in the oxidizer as in 1. above.

5. Tissues were taken from frozen storage and ground (without prior thawing) in a mortar in the presence of liquid nitrogen. Approximately 200 mg of the tissue powder was combusted in the oxidizer as before except for fat where aliquots were limited to 25 - 50 mg to permit complete combustion.

6. Various other samples (solvent extracts, aqueous column fractions, etc.) were assayed in the Triton-X system as described earlier.

Thin-layer Chromatography (TLC). Thin-layer chromatography was performed on Silica GF_{254} (E. Merck, Darmstadt) pre-coated plates of 250 micron layer thickness. Two solvent systems were employed with the following separations:

	R_f for SENCOR	DA	DK	DADK
System A - Benzene:$CHCl_3$:p-dioxane (4:3:3)	0.51	0.45	0.28	0.36
System B - $CHCl_3$:p-dioxane (9:1)	0.77	0.59	9.43	0.31

"Visualization" of separated compounds was accomplished through fluorescent quenching on the layer and/or scanning for radioactivity on a Varian/Berthold TLC scanner (Model 6000-1, Varian Instrument Company).

Gas Liquid Chromatography (GLC). GLC analyses were performed on a Beckman GC-4 gas chromatograph equipped with a helium-ionization electron capture detector. A glass U-tube column, 1/8" o.d. x 4 ft, packed with 3% PPE-20 on Supelcoport 80/100 mesh (Supelco, Incorporated) conditioned at 220°C after overnight purging with carrier gas was employed for separations.

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EXPERIMENTAL (Continued)

Operating conditions were as follows:

Gas Flow Rates (cc/min):	Helium (carrier) 30
	Helium (detector) 60
	Carbon dioxide (detector) ca. 2
Temperatures (°C):	Inlet, 210
	Column, 184
	Detector, 240
Detector Polarizing Voltage:	310 Volts D.C.

An excellent separation of SENCOR and the three known metabolites was obtained with this system. Approximate retention times were: SENCOR, 8.2 minutes; DA, 11.5 minutes; DK, 6.3 minutes and DADK, 3.1 minutes.

The electron capture detector afforded excellent sensitivity with these compounds, the minimum quantitatable amount being approximately 2.5 x 10^{-10} gm (for DADK, the least responsive).

Gel Permeation Chromatography (GPC). GPC was conducted with Sephadex G-10 polydextran beads (Pharmacia Fine Chemicals, Incorporated). The beads (100 gm) were swollen overnight in distilled water and then poured into a glass column (Chromatronix, Incorporated) 23 inches in length having a bore of 1 inch. The final bed volume of the gel was 228 ml. A continuous flow of distilled water of 1.25 ml/min was maintained through the column. The void volume of the column, as determined with Dextran Blue, was 82 ml. A "molecular size" calibration curve was prepared for the column by measuring the elution volumes of five standard compounds of decreasing molecular weight. The curve, which is shown superimposed on Figure 4, was prepared from the following elution volumes:

Standard Compound	Molecular Weight	Elution Volume
Dextran Blue	ca. 20,000	70-88 ml
Evans Blue	960	80-92 ml
Bromthymol Blue	646	120-140 ml
p-nitrophenyl Glycoside	301	288-360 ml
Methyl Red	269	660-820 ml

Fractions of the eluate were collected in an Ultro-Rak fraction collector (LKB, Incorporated).

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EXPERIMENTAL (Continued)

Ion Exthange Chromatography. Ion exchange liquid column chromatography was used for separation of radioactivity in urine ftactions taken from the GPC column. An estimate of ion-exchangeability was performed using small columns (ca. 10 ml bed volume) of Dowex-50(H^+) and Dowex-1 (CL^-) pouted in water and eluted first with water and then increasing toncentrations of HCl or NaOH respectively, up to 5.0*N*. An estimation of anion-exchangeability was performed with DEAE, AE and PAB cellulose packings in a similar manner using watet and 3*N* NH_4OH for elution.

AE cellulose was thosen for the final separation of GPC tolumn urine fractions. The protedure used with this cxchanger was as follows. One gm Celu ION AE (Nutritional Biochemicals Corporation) was slutried in distilled water, sonicated for a few minutes in a sonicator bath (Desontegrator, Ultrasonic Industrries, Incorporated) and allowed to settle for about ten minutes before detanting off the "fines." Resuspension, sonitation, settling and decantation were repeated twite more. The packing was then poured into an 8 mm i.d. x 40 mm glass tolumn (Fisher & Porter, Incorporattd), using a glass fibte filter paper pad on the glass frit support. The tolumn was filled to about 30 tm with water before addition of the slurry; the slurry was added in sevetal portions. The sample was added to the top of the column in 1 ml water and eluted with a gradient of NH_4OH. Thc tomplex gradient was achieved with a Varigrad (Buchler, Incorporated) using thtee tanks containing 50 ml each of water, 0.1*N* and 1.0*N* NH_4OH, tespettively. One ml fraetions were collected during gradient elution with the fraction collettor described earlier.

Enzyme Incubarions. Urine and tissuts were incubated with various hydtolytit and proteolytic enzymes under varying conditions dependent on the properties of the enzyme. All incubarions were performed in a watet bath shaker at 37°C for 16 - 18 hours in 0.1*N* sodium acetate buffer (urine) ot M/15 sodium phosphate buffer (tissues). Enzyme concentrations and pH's of iotubation were as follous:

Enzyme	Wt/mg or ml Substrate	Substrate	pH
9-glucuronidase	10 mg	Urine	5
Aryl-sulfatase	5 mg	Urine	5
8-glututonidase	52 µg	Liver	5
Aryl-sulfatase	24 µg	Liver	5
Trypsin	33 µg	Liver	7
Pepsin	130 µg	Liver	5
Ficin	42 µg	Liver	7
Papain	Conditions taken from reference (4)		

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EXPERIMENTAL (Continued)

Standard substrates were included in incubations with β-glucuronidase and aryl-sulfatase to insure enzyme activity. There were phenophthalein glucuronide and p-nitrophenyl sulfate which gave appropriate color reactions after enzymolysis.

Chemical Hydrolysis. Urine samples were hydrolyzed directly with HCl (final concentration 1.0N) in a boiling water bath for 100 minutes. Urine and tissue samples were similarly hydrolyzed in a steam autoclave at 121°C (15 psi). Urine and tissue samples were also hydrolyzed in the autoclave without the addition of acid or base.

Mild acid hydrolysis was accomplished in a final concentration of 0.2N HCl at 37°C while mild alkaline hydrolysis took plate in 0.047M Na_2CO_3 (pH ca. 11) at 37°C.

Combined Enzyme Digestion and Acid Hydrolysis of Tissues. One-half gram of tissue was homogenized in 10 ml M/15 phosphate buffer (pH 5) and transferred to an incubation flask with 5 ml of the same buffer containing 45 mg Pepsin (Sigma, 1:10,000) and 15 mg β-glucuronidase (Sigma, Bacterial Type II). The mixture was incubated at 37°C for 20 hours in a water bath shaker (New Brunswick Scientific Company, Incorporated) and then transferred to a 45-ml conical centrifuge tube with buffer, diluting to a final volume of 15 ml then centrifuged. Thirteen ml were extracted twice with 25 ml isopropyl ether (IPE), and the organic extracts were concentrated for TLC.

One ml concentrated HCl was added to 12 ml of the incubate and the mixture autoclaved at 121°C (15 psi) for one hour. The pH was adjusted to 5 with 3 ml 4N NaOH and, after dilution to 25 ml with buffer, IPE extraction was repeated. Aliquots were taken for radioassay after each manipulation.

Deproteinization. Various samples were deproteinized by making the sample to 0.4N with $HClO_4$ at ice bath temperature. The samples were maintained under those conditions for 15 - 30 minutes followed by neutralization with 6.2N K_2CO_3. The protein and insoluble $KClO_4$ were removed from the sample by centrifugation.

RESULTS AND DISCUSSION

Excretion Experiments

Two separate excretion experiments were performed, one used SENCOR labeled with both carbon-14 and tritium and another employing only the carbon-labeled material. In the former, a male and female rat were each given a single, oral dose of 20 mg/kg SENCOR, and urine, feces and expired gases were collected. The results of that study are shown in Table 1 in terms of excretion of radiocarbon in urine and feces and of tritium in expired gases. No radiocarbon was excreted in the expired gases, and only about 39% of the administered tritium was found in the urine and feces.

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